

## **CURRICULUM VITAE ET STUDIORUM**

### **DADOS PESSOAIS**

**Nome:** Rogerio Silveira Vilela

**Telefones de Contato:** (35) 9 9190-1009

**E-mail:** vilela.rogerio@gmail.com

**Curriculum Lattes:** <http://lattes.cnpq.br/4488210878030142>

### **FORMAÇÃO ACADÊMICA**

#### **DOUTORADO no Programa de Engenharia Hidráulica e Saneamento. Universidade de São Paulo / Escola de Engenharia de São Carlos, USP/EESC, Brasil.**

Título: PRODUÇÃO DE HIDROGÊNIO E METANO A PARTIR DE SUBPRODUTO DA INDÚSTRIA SUCROALCOOLEIRA, EM REATORES ANAERÓBIOS DE FASES SEPARADAS SOB CONDIÇÃO TERMOFÍLICA, Ano de obtenção: 2016.

Orientador: Dra. Márcia Helena Rissato Zamariolli Damianovic.

Bolsista do(a): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES, Brasil.  
Palavras-chave: Produção de Hidrogênio; Produção de Metano; Processos anaeróbios de separação de fases; Condição termofílica; Indústria Sucroalcooleira; Biologia Molecular.

Grande Área: Engenharias.

Grande Área: Engenharias / Área: Engenharia Sanitária / Subárea: Saneamento Ambiental / Grande Área: Ciências Biológicas / Área: Bioquímica / Subárea: Biologia Molecular.

Especialidade: Microbiologia Aplicada e Engenharia Sanitária.

Setores de atividade: Coleta, tratamento e disposição de resíduos; recuperação de materiais.

#### **MESTRADO no Departamento de Engenharia Hidráulica e Saneamento. Universidade de São Paulo / Escola de Engenharia de São Carlos, USP/EESC, Brasil.**

Título: REMOÇÃO DE MATÉRIA ORGÂNICA DE ÁGUAS RESIDUÁRIAS COM ELEVADA CONCENTRAÇÃO DE SULFATO PELAS VIAS SULFETOGÊNICA E METANOGÊNICA COMBINADAS, Ano de Obtenção: 2012.

Orientador: Dra. Márcia Helena Rissato Zamariolli Damianovic.

Bolsista do(a): Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq, Brasil.

Palavras-chave: Reator Anaeróbio; Tecnologia Anaeróbia; Redução de Sulfato.

Grande Área: Engenharias.

Grande Área: Engenharias / Área: Engenharia Sanitária / Subárea: Saneamento Ambiental.

Grande Área: Ciências Biológicas / Área: Microbiologia / Subárea: Biologia e Fisiologia dos Microorganismos.

Setores de atividade: Água, esgoto, atividades de gestão de resíduos e descontaminação; Coleta, tratamento e disposição de resíduos; recuperação de materiais.

**GRADUAÇÃO em Engenharia Civil – Bacharelado.**

Universidade do Estado de Minas Gerais - Campus Passos, UEMG, Brasil.

Título: EXTRAÇÃO DE AGREGADOS NATURAIS PARA A CONSTRUÇÃO CIVIL: ESTUDO DE CASO EM PLANTA DE PORTO DE AREIA NO MUNICÍPIO DE PASSOS - MG.

Orientador: Fernanda Medeiros Dutra Reis.

**GRADUAÇÃO em Ciências Biológicas – Licenciatura.**

Universidade do Estado de Minas Gerais - Campus Passos, UEMG, Brasil.

Bolsista do(a): Fundação de Amparo à Pesquisa do Estado de Minas Gerais, FAPEMIG, Brasil.

**APROVAÇÕES EM CONCURSOS PÚBLICOS**

2022

Homologação de processo seletivo para contratação de professor substituto pelo Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas Gerais (IFSULDEMINAS), Edital 155/2022.

**CURSOS COMPLEMENTARES****Informática Aplicada e Avançada para Engenharia**

ArcGIS - ESRI – curso ministrado pela Escola de Engenharia de São Carlos da Universidade de São Paulo – EESC/USP - Campus São Carlos;

AutoCAD – curso ministrado pela MAPData autorizada AutoDesk;

Revit – curso ministrado pela ImplantabIM autorizada AutoDesk;

Robot Structural Analysis – curso ministrado pela ImplantabIM autorizada AutoDesk;

FTool – Udemy Inc;

CypeCad 2019 – Udemy Inc.

**Inglês Nível Avançado**

Proficiência em Língua Inglesa - TEAP (*Test of English for Academic and Professional Purposes*).

**Informática Aplicada**

Microsoft Word;

Microsoft Excel;

MS-Project Aplicado à Construção Civil.

**FORMAÇÃO COMPLEMENTAR**

2007 - 2007

Biologia: Comportamento e Extração de Veneno. (Carga horária: 4h).

Fundação de Ensino Superior de Passos, FESP, Brasil.

2007 - 2007

Kumon - Instituto de Educação. (Carga horária: 3h). (Carga horária: 3h).

Fundação de Ensino Superior de Passos, FESP, Brasil.

2006 - 2006

Produção de Mudas: Reflorestamento de Mata ciliar. (Carga horária: 5h).  
Fundação de Ensino Superior de Passos, FESP, Brasil.

2006 - 2006

Teste de Toxicidade em Ouriço-do-mar. (Carga horária: 5h).  
Fundação de Ensino Superior de Passos, FESP, Brasil.

2006 - 2006

Biologia e Reconhecimento de Serpentes Peçonhentas. (Carga horária: 5h).  
Fundação de Ensino Superior de Passos, FESP, Brasil.

2006 - 2006

Ecotoxicidade de organismos de água doce. (Carga horária: 5h).  
Fundação de Ensino Superior de Passos, FESP, Brasil.

2006 - 2006

Teste de Toxidade com *Daphnia similis*. (Carga horária: 4h).  
Fundação de Ensino Superior de Passos, FESP, Brasil.

2006 - 2006

Macro-fotografia. (Carga horária: 5h).  
Fundação de Ensino Superior de Passos, FESP, Brasil.

2006 - 2006

Alimentação de Natural de Peixes. (Carga horária: 5h).  
Fundação de Ensino Superior de Passos, FESP, Brasil.

## ATUAÇÕES PROFISSIONAIS

**UNIVERSIDADE DE SÃO PAULO / ESCOLA DE ENGENHARIA DE SÃO CARLOS – EESC/USP, BRASIL**

Vínculo institucional

2010 - 2016

Vínculo: Bolsista (Mestrado e Doutorado), Enquadramento Funcional: Bolsista (Mestrado e Doutorado)

**MINERALI ENGENHARIA (PROFISSIONAL LIBERAL EM ENGENHARIA)**

Passos – MG; Fone: (35) 3521-9106

Atividades Desenvolvidas:

- Consultoria na área de construção civil;
- Consultoria para obtenção de licenças ambientais junto a órgãos públicos;
- Análise de afluentes e efluentes – Estação de Tratamento de Esgoto – ETE, Estação de Tratamento de Água – ETA;
- Desenvolvimento de projetos de geoprocessamento em sistemas CAD e GIS;
- Análise de dados para geoprocessamento;
- Tratamento de imagens para delimitação de áreas de risco, preservação, culturas, etc;
- Gerenciamento de clientes.

**SECRETARIA DE ESTADO DE EDUCAÇÃO DE MINAS GERAIS, SEE-MG, Brasil.**

Vínculo institucional

2007 - 2007

Vínculo: Servidor Público, Enquadramento Funcional: Professor Substituto

**UNIVERSIDADE FEDERAL DE MINAS GERAIS, UFMG, BRASIL.**

Vínculo institucional

2008 - 2008

Vínculo: Estágio, Enquadramento Funcional: Estagiário, Carga horária: 40

Outras informações

Estágio realizado no laboratório NUVELHAS no projeto Manuelzão

**PREFEITURA MUNICIPAL DE PASSOS, PMP, BRASIL.**

Vínculo institucional

2005 - 2006

Vínculo: Estágio, Enquadramento Funcional: Estagiário, Carga horária: 20

Outras informações

Atuação na área de reforço escolar, educação ambiental, desenvolvendo projetos com crianças na faixa etária de 07 à 15 anos. Programa intitulado de Jornada Ampliada.

**FUNDAÇÃO DE ENSINO SUPERIOR DE PASSOS DA UNIVERSIDADE DO ESTADO DE MINAS GERAIS, FESP-UFGM, BRASIL.**

Vínculo institucional

2008 - 2009

Vínculo: Estagiário, Enquadramento Funcional: Estágio, Carga horária: 20

Outras informações

Estagiário do Laboratório de Hidrobiologia da Universidade do Estado de Minas Gerais, grande área de Ecologia, área de ecologia de organismos bentônicos de sistemas Lênticos (reservatórios) e sua relação com a qualidade da água.

Atividades

01/2008 - 03/2009

Estágios, Laboratório de Hidrobiologia.

Estágio realizado

Estágio na área de Limnologia com ênfase em organismos bentônicos de sistemas lênticos.

03/2007 - 12/2007

Estágios, Laboratório de Hidrobiologia.

Estágio na Área de Limnologia com ênfase em organismos do Fitoplâncton e Zooplâncton

**PUBLICAÇÕES RELEVANTES****ARTIGOS COMPLETOS PUBLICADOS EM PERIÓDICOS**

1.

VILELA, R.S.; FUESS, L.T.; SAIA, F.T.; SILVEIRA, C.R.M.; OLIVEIRA, C.A.; ANDRADE, P.A.; LANGENHOFF, A.; VAN DER ZAAN, B.; COP, F.; GREGORACCI, G.B.; DAMIANOVIC, M.H.R.Z. Biofuel production from sugarcane molasses in thermophilic anaerobic structured-bed reactors. RENEWABLE & SUSTAINABLE ENERGY REVIEWS, v. 144, p. 110974, 2021.

Citações:8

2.

VILELA, ROGÉRIO; SAIA, FLÁVIA TALARICO; GREGORACCI, GUSTAVO BUENO DUARTE, RUBENS ; ANDRADE, PEDRO; VAN DER ZAAN, BAS; LANGENHOFF, ALETTE; DAMIANOVIC, MÁRCIA H.R. Z. Hydrogen production in reactors: The influence of organic loading rate, inoculum and support material. INTERNATIONAL JOURNAL OF HYDROGEN ENERGY, v. 44, p. 27259-27271, 2019.

Citações:9

3.

VILELA, ROGERIO SILVEIRA; DAMIANOVIC, MÁRCIA HELENA RISSATO ZAMARIOLLI; FORESTI, EUGENIO. Removing organic matter from sulfate-rich wastewater via sulfidogenic and methanogenic pathways. Water Science and Technology, v. 69, p. 1669-1675, 2014.

Citações:18|6

## TRABALHOS COMPLETOS PUBLICADOS EM ANAIS DE CONGRESSOS

1.

VILELA, R. S.; SAIA, F. T. ; DAMIANOVIC, M. H. R. Z. . Hydrogen and Methane production from the sugarcane industry by-product by anaerobic bioreactors with fixed-structured bed (ABFSB) two-stage process operated under thermophilic condition. In: XII Latin American Workshop and Symposium on Anaerobic Digestion, 2016, Cuzco. Hydrogen and Methane production from the sugarcane industry by-product by anaerobic bioreactors with fixed-structured bed (ABFSB) two-stage process operated under thermophilic condition, 2016.

2.

VILELA, R. S.; SAIA, F. T. ; DAMIANOVIC, M. H. R. Z. . Influence Of Organic Loading Rate, Source Of Inocula And Support Material On Hydrogen Production. In: IWA 14th World Congress on Anaerobic Digestion, 2015, Viña Del Mar. IWA 14th World Congress on Anaerobic Digestion, 2015.

3.

VILELA, R. S.; SAIA, F. T. ; DAMIANOVIC, M. H. R. Z. . Avaliação da geração de metano de água resíduária rica em sulfato em reator anaeróbio horizontal de leito fixo -RAHLF. In: Workshop Latino-Americano de Bio-Hidrogênio, 2014, São Carlos / SP. Avaliação da geração de metano de água resíduária rica em sulfato em reator anaeróbio horizontal de leito fixo - RAHLF, 2014.

4.

VILELA, R. S.; DAMIANOVIC, M. H. R. Z. ; FORESTI, E. . Biological organic matter removal and sulfate reduction from sulfate-rich wastewater in horizontal-flow anaerobic immobilized biomass reactor (HAIB) Reference. In: IWA 13th World Congress on Anaerobic Digestion, 2013, Santiago de Compostela. IWA 13th World Congress on Anaerobic Digestion, 2013.

5.

VILELA, R. S.; DAMIANOVIC, M. H. R. Z. ; FORESTI, E. . Organic matter removal from wastewater with high sulfate concentration by sulfidogenic and methanogenic combined pathways. In: IWA 13th World Congress on Anaerobic Digestion, 2013, Santiago de Compostela. IWA 13th World Congress on Anaerobic Digestion, 2013.

## **APRESENTAÇÕES DE TRABALHO**

1.  
VILELA, R. S.. Ecologia e Aplicação de Técnicas de Biologia Molecular em Reatores Anaeróbios. 2011. (Apresentação de Trabalho/Congresso).
2.  
VILELA, R. S.; STRIPARI, N. L. ; Carvalho . Caracterização da Comunidade Bentônica em Três Braços do Rio Sapucaí no Reservatório da UHE de Furnas - MG. 2008. (Apresentação de Trabalho/Congresso).
3.  
SILVA ; STRIPARI, N. L. ; VILELA, R. S. . Avaliação da Degradação do Rio São João em Áreas Agropastoris Através da Comunidade de Macroinvertebrados Bentônicos. 2008. (Apresentação de Trabalho/Congresso).
4.  
VILELA, R. S.; STRIPARI, N. L.; Carvalho. Utilização do Índice BMWP (Junqueira e Campos, 1998) em Três Braços do Rio Sapucaí da UHE de Furnas - MG. 2008. (Apresentação de Trabalho/Congresso).
5.  
SA, O. R. ; VILELA, R. S. ; Pereira ; Carvalho ; Reis ; STRIPARI, N. L. . Utilizando o Índice BMWP (Junqueira e Campos, 1998) no Reservatório da UHE de Marechal Mascarenhas de Moraes da Bacia Hidrográfica do Médio Rio Grande - MG. 2008. (Apresentação de Trabalho/Simpósio).

## **PARTICIPAÇÃO EM BANCAS**

### **PARTICIPAÇÃO EM BANCAS DE TRABALHOS DE CONCLUSÃO**

#### **Trabalhos de conclusão de curso de graduação**

1.  
SA, O.R.; VILELA, R.S.; OLIVEIRA, TCT. Participação em banca de Raquel Ferreira Machado.Impactos Ambientais Causados por Águas Residuárias de Abatedouro no Ribeirão Bocaina no Município de Passos - MG. 2011. Trabalho de Conclusão de Curso (Graduação em Engenharia Ambiental) - Universidade do Estado de Minas Gerais - Campus Passos.

## **EVENTOS**

### **PARTICIPAÇÃO EM EVENTOS, CONGRESSOS, EXPOSIÇÕES E FEIRAS**

1.  
XII Latin American Workshop and Symposium on Anaerobic Digestion. Hydrogen and Methane production from the sugarcane industry by-product by anaerobic bioreactors with fixed-structured bed (ABFSB) two-stage process operated under thermophilic condition. 2016. (Congresso).

2.  
IWA 14th World Congress on Anaerobic Digestion. 2015. (Congresso).
3.  
IWA 14th World Congress on Anaerobic Digestion. Influence of organic loading rate, source of inocula and support material on hydrogen production from sugarcane molasses in up-flow structured bed reactors operated under thermophilic condition. 2015. (Congresso).
4.  
IWA 13th World Congress on Anaerobic Digestion. 2013. (Congresso).
5.  
IWA 13th World Congress on Anaerobic Digestion. Organic matter removal from wastewater with high sulfate concentration by sulfidogenic and methanogenic combined pathways. 2013. (Congresso).
6.  
IWA 13th World Congress on Anaerobic Digestion. Biological organic matter removal and sulfate reduction from sulfate-rich wastewater in horizontal-flow anaerobic immobilized biomass reactor (HAIB). 2013. (Congresso).
7.  
II Seminário do Projeto Temático FAPESP. Remoção de matéria orgânica de águas residuárias com elevada concentração de sulfato pelas vias metanogênica e sulfetogênica combinadas. 2012. (Seminário).
8.  
II Congresso de Ecologia do Sudoeste Mineiro. Ecotoxicologia e Ecologia de Reatores Anaeróbios. 2011. (Congresso).
9.  
Obtenha Melhores Resultados Usando o ArcGIS 10 - Desktop. 2010. (Seminário).
10.  
VI Semana "A Pós-Graduação da EESC na Biblioteca".Publicar: Obrigação do Pesquisador. 2010. (Outra).
11.  
VI Semana "A Pós-Graduação da EESC na Biblioteca".Mesa Redonda - Direito Autoral e Ética na Publicação. 2010. (Outra).
12.  
VI Semana "A Pós-Graduação da EESC na Biblioteca".Dedalus: Novos Recursos para Acesso À Informação. 2010. (Outra).
13.  
Encontro Técnico dos Pesquisadores Parceiros da Estação de Hidrobiologia e Piscicultura de Furnas. 2007. (Encontro).

14.  
Semana Universitária FESP/UEMG. 2007. (Outra).
15.  
Apicultura. 2006. (Outra).
16.  
Biodiversidade e Aspectos Ecológicos de ictiofauna de Água Doce. 2006. (Outra).
17.  
Lagoas Marginais do Rio Paranapanema. 2006. (Outra).
18.  
Mexilhões Dourado: Não Dê Carona a Esse Bicho. 2006. (Outra).
19.  
Propriedades Biológicas dos Venenos e da Secreção dos Anfíbios. 2006. (Outra).
20.  
Semana Universitária FESP/UEMG. 2006. (Outra).
21.  
Sistemática, Morfologia, Biodiversidade e Ecologia de *Hymenoptera Parasitóides*. 2006. (Outra).
22.  
Técnicas Avançadas para Melhoramento Genético de *Apis Mellifera*. 2006. (Outra).
23.  
Semana Universitária FESP/UEMG. 2005. (Outra).

## BOLSAS DE ESTUDOS

### **BOLSA DE ESTUDO EM NÍVEL DE PÓS-GRADUAÇÃO**

1.  
Bolsista de Pós-Graduação Stricto Sensu (Doutorado) pela Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES. (2012-2016)
2.  
Bolsista de Pós-Graduação Stricto Sensu (Mestrado) pelo Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq. (2010-2012)

### **BOLSA DE ESTUDO EM NÍVEL DE INICIAÇÃO CIENTÍFICA**

- 1  
Bolsista de Iniciação Científica pela Fundação de Amparo à Pesquisa de Minas Gerais – FAPEMIG. (2007-2009)



**REPÚBLICA FEDERATIVA DO BRASIL  
UNIVERSIDADE DE SÃO PAULO  
ESCOLA DE ENGENHARIA DE SÃO CARLOS**

O REITOR DA UNIVERSIDADE DE SÃO PAULO,  
NO USO DE SUAS ATRIBUIÇÕES,  
CONFERE A

**ROGERIO SILVEIRA VILELA**

DE NACIONALIDADE BRASILEIRA,  
PORTADOR DA CÉDULA DE IDENTIDADE  
RG Nº MG-11.155.624 MG,  
NASCIDO EM 04 DE FEVEREIRO DE 1980  
E NATURAL DO ESTADO DE MINAS GERAIS,

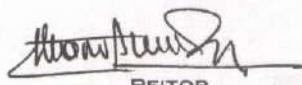
O TÍTULO DE



**DOUTOR EM CIÊNCIAS**

OBTIDO EM 02 DE DEZEMBRO DE 2016,  
NO PROGRAMA: ENGENHARIA HIDRÁULICA E SANEAMENTO,  
ÁREA DE CONCENTRAÇÃO: HIDRÁULICA E SANEAMENTO.  
E, PARA QUE POSSA GOZAR DE TODOS OS DIREITOS E  
PRERROGATIVAS LEGAIS, OUTORGA-LHE O PRESENTE DIPLOMA.

SÃO CARLOS, 03 DE JANEIRO DE 2017.



REITOR

PROF. DR. MARCO ANTONIO ZAGO



PRÓ-REITOR DE PÓS-  
GRADUAÇÃO  
PROF. DR. CARLOS GILBERTO  
CARLOTTI JUNIOR

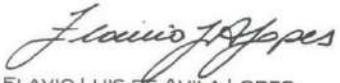
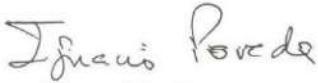


DIPLOMADO  
ROGERIO SILVEIRA VILELA

# COMPROVAÇÕES – CURRICULUM VITAE ET STUDIORUM

2

PROGRAMA: RECONHECIDO DE ACORDO COM O  
DISPOSTO NA PORTARIA MEC Nº 1077 DE  
31/08/2012, DOU DE 13/09/2012.

<p>UNIVERSIDADE DE SÃO PAULO SECRETARIA GERAL DIVISÃO DE REGISTROS ACADÊMICOS  DIPLOMA REGISTRADO SOB Nº UPG018040 PROCESSONº 2017.5.13.18.3 NOS TERMOS DO ARTIGO 48 DA LEI 9394/96. SÃO PAULO, 03 DE JANEIRO DE 2017.</p> <p> FLÁVIO LUIS DE ÁVILA LOPEZ TÉCNICO ACADÊMICO</p> <p>DE ACORDO,</p> <p> IGNÁCIO MARIA POVEDA VELASCO SECRETÁRIO GERAL PROF. DR. IGNÁCIO MARIA POVEDA VELASCO</p>
--

SECRETARIA GERAL  
DIVISÃO DE REGISTROS ACADÊMICOS  
  
O PRESENTE DOCUMENTO, EXPEDIDO PELA  
UNIVERSIDADE DE SÃO PAULO É AUTÊNTICO.  
SÃO PAULO, 03 DE JANEIRO DE 2017.



ARIOSVALDO BEZERRA DE SOUSA

ANEXO

Janus - Sistema Administrativo da Pós-Graduação

Aluno 18138/7282655-2 Página 1/2



**UNIVERSIDADE DE SÃO PAULO**

Escola de Engenharia de São Carlos

**HISTÓRICO ESCOLAR DE PÓS-GRADUAÇÃO**

**Nome:** Rogerio Silveira Vilela

**Data de Nascimento:** 04/02/1980      **Cédula de Identidade:** RG: MG-11.155.624 - MG

**Local de Nascimento:** Estado de Minas Gerais      **Nacionalidade:** Brasileira

**Graduação:** Licenciado em Ciências Biológicas - Faculdade de Filosofia de Passos - Brasil - 2009

**Mestrado:** Mestre em Ciências - Área: Hidráulica e Saneamento - Escola de Engenharia de São Carlos - Universidade de São Paulo - São Paulo - Brasil - 2012

**Título:** Doutor em Ciências

**Obtido no Programa:** Engenharia Hidráulica e Saneamento

**Área:** Hidráulica e Saneamento

**Data da Matrícula:** 17/10/2012

**Orientador:** Prof(a) Dr(a) Márcia Helena Rissato Zamariolli Damianovic

**Proficiência em Língua(s):** Inglês

**Data de aprovação no exame de qualificação:** 02/10/2014

**Título do Trabalho:** "Produção de hidrogênio e metano a partir de subproduto da indústria sucroalcooleira, em reatores anaeróbios de fases separadas sob condição termofílica"

**Data da Defesa:** 02/12/2016

**Resultado da Defesa:** Aprovado

São Carlos, 03 de Janeiro de 2017

Luis Fernando Costa Alberto

Presidente da Comissão de Pós-Graduação

03/01/17 08:17:56

Prof Associado Luis Fernando Costa Alberto  
Presidente da CPG-EESC-USP

**UNIVERSIDADE DE SÃO PAULO**

Escola de Engenharia de São Carlos

**HISTÓRICO ESCOLAR DE PÓS-GRADUAÇÃO****Nome: Rogerio Silveira Vilela**

Sigla	Nome da Disciplina	Início	Término	Créditos	Frequência	Conceito
SHS5916-1	Tratamento de Esgoto Sanitário	07/03/2013	27/06/2013	8	75	B
SHS5919-1	Processos Anaeróbios de Tratamento de Despejos	08/08/2013	21/11/2013	12	93	A
SHS5725-4	Planejamento de Experimentos e Análise de Resultados de Pesquisa na Engenharia Sanitária	11/03/2014	20/05/2014	10	90	B
SHS5722-4	Metodologia do Ensino de Engenharia	11/03/2014	24/06/2014	12	92	A

**Créditos atribuídos à Tese:****150**

São Carlos, 03 de Janeiro de 2017

Luis Fernando Costa Alberto

Presidente da Comissão de Pós-Graduação

03/01/17 08:17:56

Prof. Associado Luis Fernando Costa Alberto  
Presidente da CPG-EESC-USP



**REPÚBLICA FEDERATIVA DO BRASIL  
UNIVERSIDADE DE SÃO PAULO  
ESCOLA DE ENGENHARIA DE SÃO CARLOS**

O REITOR DA UNIVERSIDADE DE SÃO PAULO,  
NO USO DE SUAS ATRIBUIÇÕES,  
CONFERE A

**ROGERIO SILVEIRA VILELA**



DE NACIONALIDADE BRASILEIRA,  
PORTADOR DA CÉDULA DE IDENTIDADE  
RG Nº MG-11.155.624 MG,  
NASCIDO EM 4 DE FEVEREIRO DE 1980  
E NATURAL DO ESTADO DE MINAS GERAIS,  
O TÍTULO DE



**MESTRE EM CIÊNCIAS**

OBTIDO EM 28 DE SETEMBRO DE 2012,  
NO PROGRAMA: ENGENHARIA HIDRÁULICA E SANEAMENTO,  
ÁREA DE CONCENTRAÇÃO: HIDRÁULICA E SANEAMENTO.  
E, PARA QUE POSSA GOZAR DE TODOS OS DIREITOS E  
PRERROGATIVAS LEGAIS, OUTORGA-LHE O PRESENTE DIPLOMA.

SÃO CARLOS, 25 DE JULHO DE 2013.

*João Grandino Rodas*

REITOR  
PROF. DR. JOÃO GRANDINO RODAS

*Vahan Agopyan*

PRO-REITOR DE  
PÓS-GRADUAÇÃO

PROF. DR. VAHAN AGOPYAN



*Rogerio Silveira Vilela*

DIPLOMADO  
ROGERIO SILVEIRA VILELA

PROGRAMA RECONHECIDO DE ACORDO COM  
O DISPOSTO NA PORTARIA MEC Nº 1077 DE  
31/08/2012, DOU DE 13/09/2012.

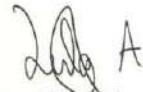
UNIVERSIDADE DE SÃO PAULO  
SECRETARIA GERAL  
DIVISÃO DE REGISTROS ACADÉMICOS

DIPLOMA REGISTRADO SOB Nº **116403**

PROCESSO Nº 2012.5.531.18.0

NOS TERMOS DO ARTIGO 48 DA LEI 9394/96.

SÃO PAULO, 8 DE NOVEMBRO DE 2013.



LEILA AÑEZ DE OLIVEIRA  
TÉCNICA PARA ASSUNTOS ADMINISTRATIVOS  
DE ACORDO.



PROF. DR. RUBENS BEÇAK  
SECRETÁRIO GERAL

SECRETARIA GERAL  
DIVISÃO DE REGISTROS ACADÉMICOS

O PRESENTE DOCUMENTO, EXPEDIDO PELA  
UNIVERSIDADE DE SÃO PAULO É AUTÊNTICO.  
SÃO PAULO, 8 DE NOVEMBRO DE 2013.

UNIVERSIDADE DE SÃO PAULO



ARIOSVALDO BEZERRA DE SOUSA

Nº 0205877

Janus - Sistema Administrativo da Pós-Graduação

Aluno 18138/7282655-1 Página 1/2



**UNIVERSIDADE DE SÃO PAULO**

Escola de Engenharia de São Carlos

**HISTÓRICO ESCOLAR DE PÓS-GRADUAÇÃO**

**Nome:** Rogerio Silveira Vilela

**Data de Nascimento:** 04/02/1980      **Cédula de Identidade:** RG: MG-11.155.624 - MG

**Local de Nascimento:** Estado de Minas Gerais      **Nacionalidade:** Brasileira

**Graduação:** Licenciado em Ciências Biológicas - Faculdade de Filosofia de Passos - Brasil - 2009

**Título:** Mestre em Ciências

**Obtido no Programa:** Engenharia Hidráulica e Saneamento

**Área:** Hidráulica e Saneamento

**Data da Matrícula:** 01/03/2010

**Orientador:** Prof(a) Dr(a) Márcia Helena Rissato Zamariolli Damianovic

**Proficiência em Língua(s):** Inglês

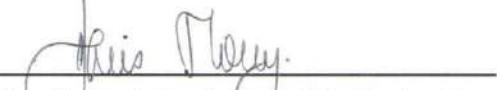
**Data de aprovação no exame de qualificação:** 28/03/2011

**Título do Trabalho:** " Remoção de matéria orgânica de águas residuárias com elevada concentração de sulfato pelas vias sulfetogênica e metanogênica combinadas"

**Data da Defesa:** 28/09/2012

**Resultado da Defesa:** Aprovado

São Carlos, 25 de Outubro de 2012

  
Presidente da Comissão de Pós-Graduação  
Prof. Titular Denis Vinicius Coury  
Presidente da CPG da EESC

25/10/12 14:30:07

# COMPROVAÇÕES – CURRICULUM VITAE ET STUDIORUM

8

Janus - Sistema Administrativo da Pós-Graduação

Aluno 18138/7282655-1 Página 2/2



UNIVERSIDADE DE SÃO PAULO

Escola de Engenharia de São Carlos

## HISTÓRICO ESCOLAR DE PÓS-GRADUAÇÃO

**Nome: Rogerio Silveira Vilela**

Sigla	Nome da Disciplina	Inicio	Término	Créditos	Frequência	Conceito
SHS5890-5	Recursos Hídricos - Aspectos Quantitativos	08/03/2010	21/06/2010	15	100	A
SHS5889-4	Recursos Hídricos - Aspectos Qualitativos	09/03/2010	22/06/2010	15	100	B
SHS5905-1	Tópicos Especiais: Gestão Sustentável da Bacia Hidrográfica Visando a Conservação dos Ciclos Naturais em Corpos de Água	02/08/2010	08/08/2010	2	100	A
SHS5732-2	Métodos para Redação e Apresentação de Seminários e Trabalhos Científicos	04/08/2010	27/10/2010	8	100	B
SHS5728-2	Algumas Continentais e suas Influências na Qualidade das Águas e Saúde Pública	05/08/2010	18/11/2010	12	93	B
SHS5742-2	Tópicos Especiais em Hidráulica e Saneamento: Sistemas de Informações Geográficas	16/08/2010	22/08/2010	2	100	A
SHS5715-4	Gerenciamento de Resíduos Sólidos	17/03/2011	07/07/2011	12	100	A
SHS5744-2	Processos Biológicos de Tratamento de Águas Residuárias (1)	02/08/2011	14/11/2011	12	87	B

**Créditos atribuídos à Dissertação:****60**

Observações:

1) Disciplina(s) cursada(s) voluntariamente pelo(a) candidato(a) após ter cumprido as exigências regulamentares.

São Carlos, 25 de Outubro de 2012

Presidente da Comissão de Pós-Graduação  
Prof. Titular Denis Vinicius Coury  
Presidente da CPG da EESC

25/10/12 14:30:07



*Universidade do Estado de Minas Gerais*

*UNIDADE PASSOS*

## **Certificado de Conclusão de Curso**

Hipólito Ferreira Paulino Neto- Diretor Acadêmico da Universidade do Estado de Minas Gerais,  
usando das atribuições que a Lei lhe confere e atendendo ao que consta dos arquivos da unidade

### ***Certifica***

Ao(a) Sr(a) Rogerio Silveira Vilela

Filiação: Oraci Gaspar Vilela e Terezinha Silveira Vilela

Identidade Registro nº MG-11.155.624

Nascido(a) aos 04 de fevereiro de 1980 em Passos (MG)

Concluiu o curso de Engenharia Civil - Bacharelado

Resolução SEDECTES N°37 de 26/03/2019, publicado em 03/04/2019. Prorrogado até 31/07/2022  
pela Portaria 06, publicada em 04/02/2022.,

tendo colado grau no dia 03/05/2022

Sendo que o diploma encontra-se em fase de registro nesta Reitoria da UEMG-BH.

E para que se produza os efeitos legais, mande expedir a presente Certidão.

Passos (MG), 20 de maio de 2022

Márcio Antônio Valadão  
Secretário de Ensino

UEMG - Unidade Passos



Hipólito Ferreira Paulino Neto  
Diretor Acadêmico

Página 1

**HISTÓRICO****Universidade do Estado de Minas Gerais**

**UNIVERSIDADE  
DO ESTADO DE MINAS GERAIS**



Recredenciada nos termos da Resolução SEDECTES nº 59, de 28 de Agosto de 2018, publicado no DOEMG em 30 de Agosto de 2018.

**Unidade Acadêmica Passos**

**Avenida Juca Stockler, 1130 - Bairro: Belo Horizonte - Cidade: Passos - Estado: MG - CEP: 37900106**

**35 - 3529 6040 - www.uemg.br**

<b>Nome:</b> Rogerio Silveira Vilela	<b>Matrícula:</b> 21-30924			
<b>Data Nascimento:</b> 04/02/1980	<b>Nacionalidade:</b> Brasileira			
<b>Identidade:</b> MG-11.155.624	<b>Data Expedição:</b> 11/02/2008	<b>Órgão de Expedição:</b> PCMG	<b>CPF:</b> 04224871637	
<b>Curso Anterior:</b> Ensino Médio	<b>Conclusão:</b> 16/07/2001			
<b>Estabelecimento:</b> Escola Estadual Professora Julia Kubitschek				
<b>Curso:</b> Engenharia Civil				
<b>Habilitação:</b> Bacharelado				
<b>Reconhecimento:</b> Resolução SEDECTES Nº37 de 26/03/2019, publicado em 03/04/2019. Prorrogado até 31/07/2022 pela Portaria 06, publicada em 04/02/2022.				
<b>Situação ENADE:</b> Dispensado de realização do ENADE, em razão do calendário trienal				
<b>Data da Situação:</b> 17/03/2022				
<b>Forma de Ingresso:</b> Transferência Interna - Reopção de Curso	<b>Ano:</b> 2019			
Ano/Sem	Componentes Curriculares	Nota	Resultado	Carga Horária
<b>1º Período</b>				
2019 / 1	Introdução à Engenharia	71,50	Aprovado	40
2019 / 1	Pré - Cálculo	73,00	Crédito	80
2019 / 1	Desenho Técnico	75,00	Crédito	40
2019 / 2	Técnicas de Edificações	100,00	Aprovado	80
2019 / 1	Projeto Integrador I	80,00	Crédito	80
2019 / 1	Formação Geral I	62,00	Aprovado	80
2019 / 1	Atividades Complementares	APTO	Aprovado	20
<b>2º Período</b>				
2019 / 1	Cálculo Diferencial e Integral I	90,00	Crédito	80
2019 / 1	Física I	73,00	Crédito	80
2019 / 1	Geometria Analítica e Álgebra Linear	70,00	Crédito	80
2019 / 1	Desenho por Computador	85,00	Aprovado	40
2019 / 1	Química Geral e Tecnológica	68,00	Crédito	80
2019 / 1	Projeto Integrador II	80,00	Crédito	40
2019 / 1	Atividades Complementares	APTO	Aprovado	20
<b>3º Período</b>				
2019 / 1	Cálculo Diferencial e Integral II	90,00	Crédito	80
2019 / 1	Mecânica Aplicada	80,00	Crédito	80
2019 / 1	Física II	73,00	Crédito	80
2019 / 1	Fenômenos de Transportes	70,00	Crédito	60
2019 / 1	Topografia I	80,00	Aprovado	80
2019 / 1	Metodologia da Pesquisa	80,00	Crédito	40
2019 / 1	Projeto Integrador III	80,00	Crédito	40
2019 / 1	Atividades Complementares	APTO	Aprovado	20
<b>4º Período</b>				
2019 / 1	Probabilidade e Estatística	70,00	Crédito	40
2019 / 1	Geologia	83,00	Crédito	60
2019 / 2	Cálculo Numérico	66,50	Aprovado	40
2019 / 1	Computação e Programação	80,00	Crédito	80
2019 / 2	Hidráulica	88,79	Aprovado	60
2019 / 2	Topografia II	60,00	Aprovado	60

# COMPROVAÇÕES – CURRICULUM VITAE ET STUDIORUM

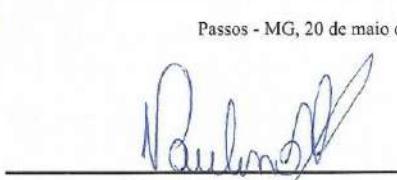
11

Nome: Rogerio Silveira Vilela				Matrícula: 21-30924
Ano/Sem	Componentes Curriculares	Nota	Resultado	Carga Horária
<b>4º Período</b>				
2020 / 1	Equações Diferenciais	73,00	Aprovado	40
2019 / 1	Projeto Integrador IV	62,50	Crédito	40
2019 / 1	Atividades Complementares	APTO	Aprovado	20
<b>5º Período</b>				
2020 / 1	Materiais de Construção I	60,00	Aprovado	80
2020 / 1	Projeto Arquitetônico	80,00	Aprovado	80
2019 / 1	Resistência dos Materiais I	80,00	Crédito	80
2020 / 2	Teoria das Estruturas I	98,00	Aprovado	80
2019 / 1	Hidrologia	75,00	Aprovado	40
2019 / 1	Projeto Integrador V	90,00	Crédito	40
2019 / 1	Atividades Complementares	APTO	Aprovado	20
<b>6º Período</b>				
2021 / 1	Teoria das Estruturas II	100,00	Aprovado	60
2020 / 1	Resistência dos Materiais II	100,00	Aprovado	80
2020 / 2	Projeto de Instalações Elétricas	67,50	Aprovado	80
2020 / 1	Geotecnia I	84,00	Aprovado	80
2019 / 2	Projeto de Instalações Hidrosanitárias	64,00	Aprovado	80
2020 / 1	Atividades Complementares	APTO	Aprovado	20
<b>7º Período</b>				
2020 / 1	Projeto de Estruturas de Concreto Armado I	81,00	Aprovado	60
2020 / 2	Materiais de Construção II	60,00	Aprovado	40
2021 / 2	Estradas, Portos e Aeroportos I	87,00	Aprovado	80
2019 / 1	Planejamento e Urbanismo	60,00	Crédito	80
2021 / 1	Geotecnia II	63,00	Aprovado	80
2019 / 1	Projeto Integrador VI	90,00	Crédito	40
2020 / 1	Atividades Complementares	APTO	Aprovado	20
<b>8º Período</b>				
2020 / 2	Projeto de Estruturas de Concreto Armado II	60,00	Aprovado	80
2020 / 2	Projeto de Estruturas Metálicas	62,00	Aprovado	40
2021 / 2	Estradas, Portos e Aeroportos II	81,00	Aprovado	80
2019 / 1	Saneamento Básico	80,00	Crédito	80
2019 / 1	Sistema de Resíduos Sólidos Urbanos	90,00	Crédito	40
2020 / 2	Materiais de Construção III	60,00	Aprovado	40
2019 / 1	Projeto Integrador VII	90,00	Crédito	40
2020 / 1	Atividades Complementares	APTO	Aprovado	20
<b>9º Período</b>				
2020 / 2	Projeto e Produção de Edifícios	73,00	Aprovado	40
2020 / 2	Projeto de Estruturas de Concreto Armado III	60,00	Aprovado	60
2019 / 1	Engenharia de Segurança	61,00	Crédito	40
2020 / 1	Projeto de Estruturas de Madeira	94,00	Aprovado	40
2020 / 2	Projeto de Edificações	78,50	Aprovado	40
2020 / 1	Sistema de Drenagem Urbana	75,00	Aprovado	40
2021 / 1	Trabalho de Conclusão de Curso I	100,00	Aprovado	40
2020 / 1	Formação Geral II	97,00	Aprovado	40
2021 / 1	Estágio Supervisionado I	APTO	Aprovado	120
2020 / 1	Atividades Complementares	APTO	Aprovado	20

*[Handwritten signature]*

# COMPROVAÇÕES – CURRICULUM VITAE ET STUDIORUM

12

Nome: Rogerio Silveira Vilela				Matrícula: 21-30924
Ano/Sem	Componentes Curriculares		Nota	Resultado
10º Período				
2021 / 1	Pontes e Concreto Protendido		70,00	Aprovado
2021 / 1	Projeto de Estruturas de Concreto Armado IV		93,00	Aprovado
2020 / 1	Avaliações e Perícias		78,00	Aprovado
2020 / 1	Fundamentos de Economia e Administração		76,00	Aprovado
2020 / 2	Orçamento, Planejamento e Gerenciamento na Construção Civil		72,00	Aprovado
2021 / 2	Trabalho de Conclusão de Curso II		90,00	Aprovado
2021 / 2	Estágio Supervisionado II		APTO	Aprovado
2020 / 2	Atividades Complementares		APTO	Aprovado
Optativas				
2021 / 2	Optativa I - Alvenaria Estrutural		70,00	Aprovado
2020 / 2	Optativa II - Tópicos Especiais em Engenharia		75,00	Aprovado
2019 / 1	Optativa I - Tópicos Especiais de Engenharia		90,00	Crédito
Carga Horária Total do Curso:				4420 horas
<b>Total da carga horária cursada: 4460 horas/aula</b>				
<b>Coeficiente de Rendimento Acadêmico do Curso: 79</b>				
<b>Observações:</b> Créditos obtidos do curso de Engenharia Ambiental - Bacharelado / Ciências Biológicas - Licenciatura - UEMG/Passos.  Optativa I - Tópicos Especiais de Engenharia com ênfase em : - Tópicos Especiais em Gestão Sustentável da Bacia Hidrográfica Visando a Conservação dos Ciclos Naturais em Corpos D' água . - Tópicos Especiais em Hidráulica e Saneamento : Sistemas de Informações Geográficas  Optativa II - Tópicos Especiais de Engenharia Civil com ênfase em Projetos de Fundações				
<b>TRABALHO MONOGRÁFICO DE CONCLUSÃO DE CURSO</b>				
<b>Título:</b> " Extração de Agregados Naturais para a Construção Civil: Estudo de Caso em Planta de Porto de Areia no Município de Passos - MG. "				
<b>Conclusão do Curso:</b> 17/03/2022		<b>Colação de Grau:</b> 03/05/2022	<b>Data de Expedição do Diploma:</b> 20/05/2022	
Passos - MG, 20 de maio de 2022				
 Secretaria Acadêmica		 Diretoria Acadêmica		
<i>Este documento, emitido pela Secretaria Acadêmica, não contém emendas, rasuras e nem alterações que utilizam outra ferramenta, exceto as assinaturas que devem ser à caneta.</i>				



Fundação de Ensino Superior de Passos  
Faculdade de Filosofia de Passos

O Presidente do Conselho Curador da FESP - Unidade Associada à Universidade do Estado de Minas Gerais - UEMG, conforme Lei Nº 18.384 de 15/09/2009, Prof. Fábio Pimenta Esper Kallas e o Diretor da Faculdade de Filosofia de Passos Prof. José de Paula Silva, no uso de suas atribuições e tendo em vista a conclusão da Graduação em Ciências Biológicas, em 3 de fevereiro de 2009, conferem o título de Licenciado em Ciências Biológicas a

**Ruyerin Silveira Villela**

Cédula de Identidade nº MG-11.155.624 SSP/MG, natural de Passos, Estado de Minas Gerais, nascido a 4 de fevereiro de 1980, de nacionalidade brasileira, e outorgam-lhe o presente Diploma, a fim de que possa gozar de todos os direitos e prerrogativas legais.

Passos-MG, 16 de setembro de 2009

Prof. Fábio Pimenta Esper Kallas  
Presidente do Conselho Curador

Ruyerin  
Diplomado

Prof. José de Paula Silva  
Diretor PAFIPA



FACULDADE DE FILOSOFIA DE PASSOS RECONHECIDA PELO DECRETO N° 66.535/70
CURSO DE CIÊNCIAS BIOLÓGICAS – (Licenciatura Plena)
RECONHECIDO PELO DECRETO N° 43.179 – 07.02.2003
RENOVAÇÃO DO RECONHECIMENTO: DECRETO DE 13/03/08
Diário Oficial de 14.03.2008

<b>UNIVERSIDADE DO ESTADO DE MINAS GERAIS</b> <b>UEMG – REITORIA</b>
Credenciada conforme o inciso IV, Art. 10, da Lei Federal nº 9.394/96, nos termos do Decreto Estadual nº 40.359, de 28 de Abril de 1999.
Diploma registrado nos termos do parágrafo 1º, Art. 48 da Lei nº 9.394, (Lei de Diretrizes e Bases da Educação Nacional), sob o nº <u>02/0</u> Lvr. <u>Ciências</u> Fis. <u>27</u> Processo nº <u>016.41.3195/09</u>
Belo Horizonte, <u>05/12/10</u>
<u>Eduardo Francisco Freire Belli, PRes</u>
Responsável pelo Registro
<u>Eduardo Francisco Freire Belli, PRes</u>
P/ Reitora de Ensino e Extensão





**Universidade do Estado de Minas Gerais**  
**Fundação de Ensino Superior de Passos**

Criada pela Lei Estadual nº 2.933/63 - Estatuto aprovado pelo Decreto Estadual nº 16.998/75  
C.G.C.(MF) 23.273.204/0001-00 - Insc. Est. Isenta



Entidade Mantenedora das Faculdades de  
Administração, Agronomia, Comunicação Social, Direito, Educação Física, Enfermagem  
Engenharia, Filosofia, Moda, Nutrição, Serviço Social, Sistema de Informação

Campus de Passos

**Histórico Escolar**

**FACULDADE DE FILOSOFIA DE PASSOS**

**Curso de Licenciatura Plena em CIÊNCIAS BIOLÓGICAS**

Nome:				Matrícula:			
ROGERIO SILVEIRA VILELA				20030448			
Pai:				Mãe:			
Oraci Gaspar Vilela				Terezinha Silveira Vilela			
Natural:				Data Nasc.:	C.M.:	C.I.:	
Passos - MG				04/02/1980	13.142.216768.7	MG-11.155.624 SSP/MG	
Escola Segundo Grau:				Local:	U.F.: Ano:		
Escola Estadual " Profª Júlia Kubitschek "				Passos	MG 2001		
Estabelecimento Vestibular:				Mês/Ano:		Total de Pontos:	
FESP - Fundação de Ensino Superior de Passos				012004		67,5	

Disciplinas	C.H.	Freq	M. Final	Ano	Sem	Situação Final
<b>Período 1</b>						
Química	72	72	7,50	2002	1	Crédito
Matemática	72	72	9,00	2004	1	Crédito
Citologia	108	108	6,00	2004	1	Crédito
Ecologia I	72	72	7,75	2004	1	Crédito
Elementos de Anatomia Humana	72	72	8,79	2004	1	Crédito
Inglês	36	36	9,00	2004	1	Crédito
Prática de Formação I (Prát. Ensino - Química e Bioquímica)	18	18	7,00	2007	1	Aprovado
<b>Período 2</b>						
Biofísica	54	54	6,00	2004	2	Crédito
Bioquímica	108	108	8,50	2005	2	Crédito
Histologia e Embriologia	108	108	6,50	2004	2	Crédito
Pesquisa e Produção de Conhecimento	36	36	7,25	2004	2	Crédito
Microbiologia	72	72	6,50	2005	1	Crédito
Informática Aplicada	36	36	8,00	2002	1	Crédito
Prática de Formação II (Prática de Ensino de Microscopia)	18	18	8,75	2007	2	Aprovado
<b>Período 3</b>						
Zoologia I	72	108	6,00	2005	2	Crédito
Genética	72	72	7,10	2005	1	Crédito
Biologia Molecular e Engenharia Genética	72	72	7,75	2005	1	Crédito
Bioestatística	72	72	7,00	2005	1	Crédito
Botânica I	72	72	6,00	2005	1	Crédito
Prática de Formação III (Prát. Ensino - Botânica e Genética)	72	64	6,50	2008	1	Aprovado
<b>Período 4</b>						
Botânica II	72	72	6,75	2005	2	Crédito
Fisiologia Geral e Comparada	90	90	6,00	2005	2	Crédito
Parasitologia	72	72	6,30	2005	2	Crédito
Entomologia	72	72	7,00	2005	2	Crédito
Fisiologia Humana	54	72	7,60	2005	2	Crédito
Imunologia	36	36	7,50	2005	2	Crédito
Prática de Formação IV (Prát. Ensino - Parasitologia)	72	78	8,50	2007	2	Aprovado
Estágio Curricular Supervisionado I	80	80		2007	2	Cumpriu
Atividades Acadêmico - Científico - Culturais	40	40		2007	2	Cumpriu
<b>Período 5</b>						
Zoologia II	90	96	7,40	2006	1	Aprovado
Botânica III	90	95	7,15	2006	1	Aprovado

# COMPROVAÇÕES – CURRICULUM VITAE ET STUDIORUM

16

<b>Nome/Matrícula:</b>	ROGERIO SILVEIRA VILELA		20030448	25/09/2009 16:33:16	
	<b>Disciplinas</b>	<b>Carg</b>	<b>Frequ</b>	<b>Méd Fin</b>	<b>Ano</b>
Ecologia II	90	72	6,50	2005	1
Evolução	72	76	6,75	2006	1
Legislação Ambiental	36	34	6,25	2006	1
Prática de Formação V (Prát. Ensino - Microbiologia e Ecologia)	72	56	7,00	2006	1
Estágio Curricular Supervisionado II	80	80		2006	1
Atividades Acadêmico - Científico - Culturais	40	40		2006	1
<b>Período 6</b>					
Geologia e Paleontologia	72	60	8,30	2006	2
Zoologia Aplicada	72	56	7,25	2006	2
Estrutura e Funcionamento da Educação Básica	54	51	8,50	2006	2
Fundamentos da Educação	72	62	7,00	2006	2
Ecologia de Populações	72	72	7,00	2006	2
Prática de Formação VI (Prát. Ensino - Educação Ambiental)	72	69	7,25	2006	2
Estágio Curricular Supervisionado III	80	80		2006	2
Atividades Acadêmico - Científico - Culturais	40	40		2006	2
<b>Período 7</b>					
Botânica Econômica	54	54	7,10	2007	1
Psicologia da Educação	72	72	8,75	2007	1
Didática, Avaliação e Teorias Pedagógicas	72	70	8,00	2007	1
Problemas de Ecologia Teórica	72	80	7,00	2007	1
Limnologia	90	105	7,50	2007	1
Prática de Formação VII (Prát. Pesq. Pedagógica em Ciências)	72	70	8,00	2007	1
Estágio Curricular Supervisionado IV	80	80		2007	1
Atividades Acadêmico - Científico - Culturais	40	40		2007	1
<b>Período 8</b>					
Agroecologia	18	18	8,00	2008	2
Ecologia Aplicada	108	36	6,50	2007	2
Patologia - Optativa	36	36	8,30	2007	2
Ecologia Vegetal	54	54	7,65	2005	2
Prática de Formação VIII (Prát. Pesq. Pedagógica em Biologia)	90	69	7,50	2007	2
Estágio Curricular Supervisionado V	80	80		2007	2
Atividades Acadêmico - Científico - Culturais	40	40		2007	2

*Data Colação de Grau:* 03/02/2009

*Data da Expedição do Diploma:* 16/09/2009

*Observações:* Transferência de Ciências Biológicas (Bacharelado) para Ciências Biológicas (Licenciatura) no 1º Semestre de 2006.

Complementação de Carga Horária: 018 Horas/aula em Ecologia II.

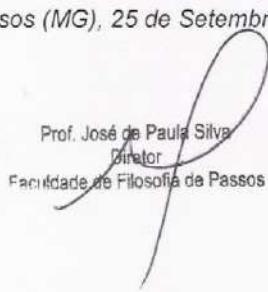
Situação Regular junto ao Exame Nacional de Desempenho dos Estudantes - ENADE 2008, tendo atendido ao que preceitua o parágrafo 5º, do Art. 5º, da Lei Nº 10.861, de 14 de abril de 2004.

*Emitido por:* \_\_\_\_\_

Passos (MG), 25 de Setembro de 2009

SECRETARIA GERAL DE REGISTRO ACADÉMICO

  
*Prof. Esdras Azarias de Campos*  
**Secretário**

  
*Prof. José de Paula Silva*  
**Diretor**  
Faculdade de Filosofia de Passos

## DIÁRIO OFICIAL DA UNIÃO - Seção 3

## EDITAL Nº 431, DE 30 DE JUNHO DE 2022

A DIRETORA GERAL DO CÂMPUS CARAGUATATUBA DO IFSP, no uso de suas atribuições delegadas pela Portaria nº 3.790, de 23/11/2018, torna pública a classificação do resultado final do Processo Seletivo Simplificado para contratação de professor substituto de que trata o Edital nº 270, de 19/05/2022, publicado no DOU em 20/05/2022 realizado no Câmpus CARAGUATATUBA, referente às seguintes áreas/disciplinas:

Área/Disciplina: INFORMATICA

Classificação	Nome	Pontuação
1º	ANDERSON ROBERTO DEIZEPE	116,0
2º	ARUAN DE LARA MORAES ALVES	83,9
3º	FELIPE SOARES PINTO DE MORAES	80,7
4º	DHIEGO HENRIQUE BALTHAZAR DE SOUZA	74,7
5º	ELKE FABIANA ALMEIDA SANTOS	69,0

JULIANA BARBARA MORAES

## INSTITUTO FEDERAL DE EDUCAÇÃO, CIÉNCIA E TECNOLOGIA DO SERGIPE

## AVISO DE LICITAÇÃO

## PREGÃO ELETRÔNICO Nº 50/2022 - UASG 158134

Nº Processo: 23290000155202247. Objeto: Aquisição de material permanente e de consumo de audiovisual para o Instituto Federal de Educação, Ciéncia e Tecnologia de Sergipe.. Total de Itens Licitados: 108. Edital: 30/06/2022 das 09h00 às 12h00 e das 13h00 às 17h00. Endereço: Rua Francisco Portugal , Nº 150, Salgado Filho - Aracaju/SE ou <https://www.gov.br/compras/edital/158134-5-00050-2022>. Entrega das Propostas: a partir de 30/06/2022, às 09h00 no site [www.gov.br/compras](http://www.gov.br/compras). Abertura das Propostas: 14/07/2022 às 09h00 no site [www.gov.br/compras](http://www.gov.br/compras). Informações Gerais: .

RUTH SALES GAMA DE ANDRADE  
Reitora

(SIASGnet - 27/06/2022) 158134-26423-2022NE999999

## AVISO DE LICITAÇÃO

## PREGÃO ELETRÔNICO Nº 44/2022 - UASG 158134

Nº Processo: 23832000010202271. Objeto: Aquisição de materiais permanentes para suprir as necessidades para oferto do Curso Técnico em Segurança do Trabalho do IFS. Total de Itens Licitados: 19. Edital: 30/06/2022 das 08h00 às 12h00 e das 13h00 às 17h00. Endereço: Av. Jorge Amado, 1551, Loteamento Garcia, Bairro Jardins, - Aracaju/SE ou <https://www.gov.br/compras/edital/158134-5-00044-2022>. Entrega das Propostas: a partir de 30/06/2022 às 08h00 no site [www.gov.br/compras](http://www.gov.br/compras). Abertura das Propostas: 13/07/2022 às 09h00 no site [www.gov.br/compras](http://www.gov.br/compras). Informações Gerais: .

DIANA INGRID PORTO FONTES CANUTO  
Pregoeira

(SIASGnet - 22/06/2022) 158134-26423-2022NE999999

INSTITUTO FEDERAL DE EDUCAÇÃO, CIÉNCIA E TECNOLOGIA DO SERTÃO PERNAMBUCANO  
PRÓ-REITORIA DE ORÇAMENTO E ADMINISTRAÇÃO  
DIRETORIA DE LICITAÇÕES

## EXTRATO DE DISPENSA DE LICITAÇÃO Nº 3/2022 - UASG 158149

Nº Processo: 2341800116202266 . Objeto: Contratação de empresa especializada em prestação de serviço de limpeza e conservação com dedicação exclusiva de mão de obra para campus salvador do Instituto Federal da Sertão Pernambucano. Total de Itens Licitados: 00013. Fundamento Legal: Art. 24º, Inciso IV da Lei nº 8.666 de 21/06/1993.. Justificativa: Justifica-se pela emergência da contratação (Lei 8.666/93, Art. 24, IV) para atender as necessidades precíprias do campus. Declaração de Dispensa em 28/06/2022. JEAN CARLOS COELHO DE ALENCAR. Pró-reitor de Administração e Orçamento. Ratificação em 28/06/2022. MARIA LEOPOLDINA VERAS CAMELO. Reitora. Valor Global: R\$ 149.813,40. CNPJ CONTRATADA : 08.794.171/0001-41 SOLONTECSERVICOS DE LIMPEZA E TRANSPORTES EIRELI.

(SIDEC - 29/06/2022) 158149-26430-2022NE000001

## INSTITUTO FEDERAL DE EDUCAÇÃO, CIÉNCIA E TECNOLOGIA DO SUDESTE DE MINAS GERAIS

CAMPUS RIO POMBA  
PRÓ-REITORIA DE ADMINISTRAÇÃO

## EXTRATO DE CONTRATO Nº 32/2022 - UASG 158123 - IF SUDESTE MG

Nº Processo: 23223.003295/2021-90. Pregão Nº 21/2002. Contratante: INSTITUTO FED CIENCIA TECNOL SUDESTE MG. Contratado: 04.947.516/0001-07 - ETERA CONSTRUÇOES E ISOLAMENTOS LTDA.. Objeto: Contratação de empresa para prestação de serviços de manutenção predial preventiva e corretiva e realização de serviços de engenharia diversos sob demanda para os campi barbacena, manhuaçu, campi avançados bom sucesso, cataguases, ubá e reitoria do if sudeste mg. Fundamento Legal: LEI 10.520 / 2002 - Artigo: 1. Vigência: 22/06/2022 a 22/06/2023. Valor Total: R\$ 1.045.033,74. Data de Assinatura: 21/06/2022.

(COMPRASNET 4.0 - 29/06/2022).

## EXTRATO DE TERMO ADITIVO

Extrato de Termo Aditivo 06/2022

PROCESSO: 23222.001292/2021-21

OBJETIVO: Prorrogação de Contrato

CONTRATANTE: Campus Rio Pomba

CONTRATADO: CESAR AUGUSTO CANESCHI

ÁREA: Ciéncia e Tecnologia de Alimentos

CARGA HORÁRIA: 40 horas

VIGÊNCIA: 18/06/2022 a 17/06/2023

## AVISO DE RETIFICAÇÃO

No EXTRATO DE CONTRATO Nº 41/2022 - UASG 158123 - IF SUDESTE MG; Contratante: INSTITUTO FED CIENCIA TECNOL SUDESTE MG - CAMPUS MURIAE; Contratado: 10.427.965/0001-19 - INSTITUTO INTERAMERICANO DE DESENVOLVIMENTO HUMANO - BE; Objeto: Contratação de serviços continuados de limpeza, com disponibilização de mão de

ISSN 1677-7069

Nº 122, quinta-feira, 30 de junho de 2022

obra em regime de dedicação exclusiva, publicado no Diário Oficial da União em 28/06/2022, Edição: 120, Seção: 3, Página: 76, onde se lê: Nº Processo: 23225.000335/2021-21, Iota-sé: Nº Processo: 23232.000531/2022-98; onde se lê: Pregão Nº 3/2021, Iota-sé: Pregão Nº 26/2022; onde se lê: Vigência: 25/03/2022 a 04/08/2022, Iota-sé: Vigência: 30/06/2022 a 30/06/2025. Ficam mantidas as demais informações.

(COMPRASNET 4.0 - 28/06/2022).

## INSTITUTO FEDERAL DE EDUCAÇÃO, CIÉNCIA E TECNOLOGIA DO SUL DE MINAS GERAIS

## EXTRATO DE TERMO ADITIVO Nº 2/2022 - UASG 158137 - IF DO SUL DE MG

Número do Contrato: 9/2021.

Nº Processo: 23343.002326/2020-48.

Regime Diferenciado de Contratações, Nº 6/2020. Contratante: INST.FED.DE EDUC.,CIENC.E TEC.,DO SUL DE MG. Contratado: 03.975.929/0001-24 - MÉTODO MATERIAIS DE INCÊNDIO E HIDRÁULICOS EIRELI. Objeto: 1. Acrescer, ao valor inicial do contrato, 23,7938913476% (dois três vírgula sete nove três oito nove um três quatro sete seis por cento), o que corresponde a R\$ 80.157,92 (oitenta mil, cento e cinquenta e sete reais e noventa e dois centavos);

2. suprir, do valor inicial do contrato, 13,7193776679% (um três vírgula sete um nove três sete seis seis seis sete nove por cento), o que corresponde a R\$ 46.218,45 (quarenta e seis mil e duzentos e dezóto reais e quarenta e cinco centavos). Vigência: 30/06/2022 a 09/02/2023. Valor Total Atualizado do Contrato: R\$ 336.884,45. Data de Assinatura: 29/06/2022.

(COMPRASNET 4.0 - 29/06/2022).

## EXTRATO DE TERMO ADITIVO Nº 1/2022 - UASG 158137 - IF DO SUL DE MG

Número do Contrato: 18/2021.

Nº Processo: 23343.001724/2021-28.

Pregão, Nº 16/2021, Contratante: INST.FED.DE EDUC.,CIENC.E TEC.,DO SUL DE MG. Contratado: 05.197.047/0001-00 - TL PUBLICIDADE E ASSESSORIA LTDA. Objeto: Prorrogação do prazo de vigência do contrato por mais 12 (doze) meses, de 15/08/2022 a 15/08/2023. Vigência: 15/08/2022 a 15/08/2023. Valor Total Atualizado do Contrato: R\$ 17.900,00. Data de Assinatura: 29/06/2022.

(COMPRASNET 4.0 - 29/06/2022).

## EDITAL Nº 192/2022 - GAB/IFSLDEMINAS

## HOMOLOGAÇÃO DE PROCESSO SELETIVO

O REITOR DO INSTITUTO FEDERAL DE EDUCAÇÃO, CIÉNCIA E TECNOLOGIA DO SUL DE MINAS GERAIS, no uso de suas atribuições legais, torna público e homologa o resultado final do PROCESSO SELETIVO SIMPLIFICADO para contratação de professor substituto, que trata o Edital Nº 155/2022, de 01.06.2022, referente à(s) seguinte(s) área(s)/disciplina(s):

Área: Engenharia Civil - Campus Pouso Alegre:

Nome do Candidato	Pontuação	Classificação
ROGERIO EDUARDO SOUZA DE ALMEIDA DIAS	90	1º
ADRIANA PETTO DE ALMEIDA SILVA CASTRO	80	2º
<b>ROGERIO SILVEIRA VILELA</b>	<b>60</b>	<b>3º</b>
GUSTAVO SOARES SANTOS	59	4º
ALINE DA SILVA DE MORAES	51	5º
ALAN HENRIQUE VICENTINI	50	6º
MATHEUS FRANCISCO DA SILVA	38	7º
JOSIMARA APARECIDA DA SILVA	37	8º
IGOR FERNANDES CAMPOS	35	9º
JOAO MARCIO SALES SIQUEIRA	35	10º

MARCELO BREGAGNOLI

## EDITAL Nº 187/2022 - GAB/IFSLDEMINAS

## HOMOLOGAÇÃO DE PROCESSO SELETIVO

O REITOR DO INSTITUTO FEDERAL DE EDUCAÇÃO, CIÉNCIA E TECNOLOGIA DO SUL DE MINAS GERAIS, no uso de suas atribuições legais, torna público e homologa o resultado final do PROCESSO SELETIVO SIMPLIFICADO para contratação de professor substituto, que trata o Edital Nº 156/2022, de 01.06.2022, referente à(s) seguinte(s) área(s)/disciplina(s):

Área: Matemática - Campus Pouso Alegre

Nome do Candidato	Pontuação	Classificação
GILBERTO CASTRANO CUNHA DE ANDRADE	100	1º
EUNICE PALMA	76	2º
SOLANGE CRISTINA RAIMUNDO ALVES	75	3º
JOAO PAULO BUENO	75	4º
ANDRE RENAN PEREIRA	72	5º

MARCELO BREGAGNOLI

## EDITAL Nº 190/2022 - GAB/IFSLDEMINAS

## HOMOLOGAÇÃO DE PROCESSO SELETIVO

O REITOR DO INSTITUTO FEDERAL DE EDUCAÇÃO, CIÉNCIA E TECNOLOGIA DO SUL DE MINAS GERAIS, no uso de suas atribuições legais, torna público e homologa o resultado final do PROCESSO SELETIVO SIMPLIFICADO para contratação de professor substituto, que trata o Edital Nº 157/2022, de 01.06.2022, referente à(s) seguinte(s) área(s)/disciplina(s):

Área: Administração - Campus Passos

Nome do Candidato	Pontuação	Classificação
STEPHANIE DUARTE ESTEBAN	79	1º
EDWARD BERNARD BASTIAAN DE R. Y. RIVERA	76	2º
LUCAS COUTO MOREIRA	72	3º
JOEL JOAQUIM DE SANTANA FILHO	70	4º
DINY GABRIELLY DE MIRANDA MARTINS	60	5º

MARCELO BREGAGNOLI



## Removing organic matter from sulfate-rich wastewater via sulfidogenic and methanogenic pathways

Rogerio Silveira Vilela, Márcia Helena Rissato Zamariolli Damianovic and Eugenio Foresti

### ABSTRACT

The simultaneous organic matter removal and sulfate reduction in synthetic sulfate-rich wastewater was evaluated for various chemical oxygen demand (COD)/sulfate ratios applied in a horizontal-flow anaerobic immobilized sludge (HAIS) reactor. At higher COD/sulfate ratios (12.5 and 7.5), the removal of organic matter was stable, likely due to methanogenesis. A combination of sulfate reduction and methanogenesis was clearly established at COD/sulfate ratios of 3.0 and 1.9. At a COD/sulfate ratio of 1.0, the organic matter removal was likely influenced by methanogenesis inhibition. The quantity of sulfate removed at a COD/sulfate ratio of 1.0 was identical to that obtained at a ratio of 1.9, indicating a lack of available electron donors for sulfidogenesis. The sulfate reduction and organic matter removal were not maximized at the same COD/sulfate ratio; therefore, competitive inhibition must be the predominant mechanism in establishing an electron flow.

**Key words** | anaerobic reactor, biological treatment, COD/sulfate ratio, methanogenesis, sulfidogenesis

Rogerio Silveira Vilela  
Márcia Helena Rissato Zamariolli Damianovic  
Eugenio Foresti (corresponding author)  
Department of Hydraulic and Sanitation Engineering,  
Escola de Engenharia de São Carlos,  
Universidade de São Paulo,  
Av. Trabalhador São-Carlense,  
400, São Carlos – São Paulo,  
Brazil  
E-mail: eforesti@sc.usp.br

### INTRODUCTION

The presence of sulfate in wastewater that has been subjected to anaerobic treatment processes can interfere with methanogenic processes in different ways, including competing for electron donors and producing sulfide and CO<sub>2</sub> through complete oxidation via sulfate-reducing bacteria (SRB) or acetate and sulfide via SRB that are incomplete oxidizers (Colleran *et al.* 1995; Omil *et al.* 1998; Damianovic & Foresti 2009). Other effects include stimulation (Zehnder & Wuhrmann 1977; Speece 1983) due to sulfur's role as a macronutrient for MA (methanogenic archaea); toxicity (Muyzer & Stams 2008) caused by a high concentration of sulfide in the liquid phase; and syntrophy (Bryant *et al.* 1967) resulting from incomplete SRB-mediated oxidation, which produces acetate, the main substrate for acetoclastic methanogens.

In the presence of sulfate without other electron acceptors, the electron flux in organic matter degradation is directed toward sulfidogenesis, methanogenesis or a combination of the two (Lens *et al.* 1998). The chemical oxygen demand (COD)/sulfate ratio is deemed the most important parameter driving the electron flux, as recently studied by several authors (Mulopo *et al.* 2011; Subtil *et al.* 2011; Cao

*et al.* 2012; Li *et al.* 2012). For COD/sulfate ratios equal to or below 0.67, the organic matter is completely removed via sulfidogenesis. The reactor configuration, the wastewater composition, the operating time and the sulfate concentration are also important factors affecting the ability of SRB to outcompete MA. At higher COD/sulfate ratios, the complete removal of organic matter is only possible through methanogenesis (Lens *et al.* 1998).

However, the long-term performance of immobilized biomass reactors that treat wastewater subjected to different COD/sulfate ratios remains uncertain. The literature contains controversial information regarding the extent of competition for electron donors, the factors affecting the acclimatization of the MA to sulfide and the influence of the reactor configuration on the processes.

This study evaluates the performance of a horizontal-flow anaerobic immobilized sludge (HAIS) reactor for the treatment of sulfate-rich wastewater subjected to different COD/sulfate ratios. Special attention is given to evaluating the electron flux, which supports methanogenesis and sulfidogenesis at varying COD/sulfate ratios.

## METHODS

The bench-scale HAIS reactor, with a total volume of 1,991 mL, consisted of a 1-m-long tube with a 5-cm diameter and four ports equally spaced (20 cm) along its length for sampling purposes. The HAIS reactor was filled with polyurethane foam matrices for biomass immobilization. These characteristics defined the fluid dynamics as a non-ideal plug-flow system. The reactor was installed inside a 30 °C temperature-controlled chamber (Figure 1).

The inoculum was the sludge taken from an up-flow anaerobic sludge blanket (UASB) reactor treating poultry slaughterhouse. The granular sludge was macerated before immobilization in polyurethane foam matrices, and inoculation was according to procedures adopted by Foresti *et al.* (1995). The reactor was subjected to synthetic wastewater containing 30 mg L<sup>-1</sup> of sulfate (COD/sulfate = 50 mg L<sup>-1</sup>) for 2 weeks. At the end of this startup period, the reactor presented high COD removal efficiency and the operation was performed through six stages.

The hydraulic retention time was set at 12 ± 1 h with a constant flow maintained by a peristaltic pump at a flow rate of 60 mL h<sup>-1</sup>. Anaerobic sludge (total volatile solids of 500 mg L<sup>-1</sup>) from the UASB reactor for the treatment of poultry slaughterhouse wastewater was used as the inoculum. The HAIS reactor was operated for 216 days.

The HAIS reactor was supplied with synthetic sulfate-rich wastewater and ethanol as the carbon source and electron donor (Table 1). The operating conditions comprised

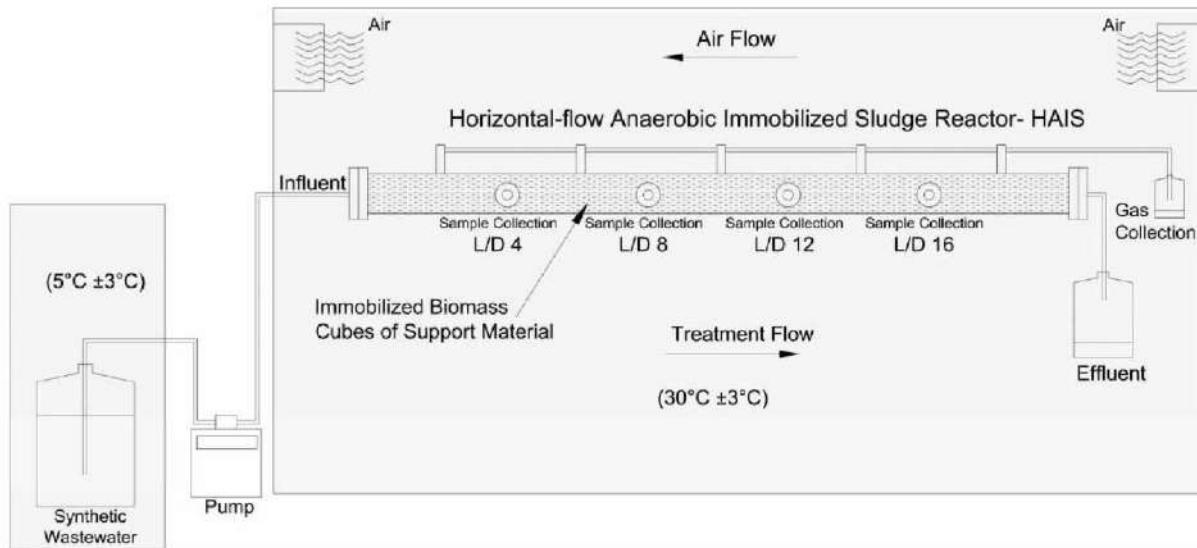
six distinct stages in which the organic matter concentration was fixed while the sulfate concentration gradually increased (through the addition of Na<sub>2</sub>SO<sub>4</sub>), thus decreasing the COD/sulfate ratio (Table 2).

Ethanol (CH<sub>3</sub>CH<sub>2</sub>OH) was chosen as an organic electron donor because it is easy to manipulate and its performance in combined sulfidogenesis and methanogenesis is well understood. Sodium hydroxide (NaOH) was added to maintain a pH between 7.0 and 8.0 to minimize the toxic effects of the sulfide (Hulshoff Pol *et al.* 1998).

The reactor performance was evaluated based on the influent and effluent analyses of sulfate, COD, volatile fatty acids (VFA) and sulfide according to APHA (2005). The concentration gradients of COD, sulfate and sulfide along the reactor's length were determined in duplicate samples taken twice weekly at the end of every experimental stage except stage 1.

The sulfate was determined via ion chromatography using a Dionex® chromatograph, model ICS-5000 (Thermo Scientific™, Sunnyvale, CA, USA), and the results were obtained using Dionex® Chromeleon Instrumental Control 6.8 software. The sulfide in the liquid phase was determined using the colorimetric method according to APHA (2005). For COD determination, sulfide was previously removed by precipitation with zinc acetate.

The electron fluxes in the organic matter removal via sulfidogenesis and methanogenesis were calculated by assuming that the electron donor was completely oxidized by SRB (Equations (1)–(3)). The fraction of organic matter



**Figure 1** | Scheme of the bench-scale HAIS reactor.

**Table 1** | Synthetic wastewater composition

Constituent	Stock solution (g L <sup>-1</sup> )	Volume added (mL L <sup>-1</sup> )
Macronutrient solution 1 (Visser <i>et al.</i> 1993)		
NH <sub>4</sub> Cl	15.00	1.00
KH <sub>2</sub> PO <sub>4</sub>	17.50	
Macronutrient solution 2 (Visser <i>et al.</i> 1993)		
KCl	27.00	0.03
MgCl <sub>2</sub> ·6H <sub>2</sub> O	15.00	
Solution for pH control		
NaOH	80.00	1.50
Micronutrient/metal solution		
C <sub>6</sub> H <sub>9</sub> NO <sub>6</sub>	12.80	1.00
FeCl <sub>3</sub> ·6H <sub>2</sub> O	01.35	
MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.100	
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.024	
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.100	
ZnCl <sub>2</sub>	0.100	
CuCl <sub>2</sub> ·2H <sub>2</sub> O	0.025	
H <sub>3</sub> BO <sub>3</sub>	0.010	
NaMoO <sub>4</sub> ·H <sub>2</sub> O	0.024	
NaCl	01.00	
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	0.026	
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.120	
Vitamin solution (Touzel & Albagnac 1983)		
C <sub>10</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub> S	0.009	0.5
C <sub>10</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub> S	0.009	
C <sub>12</sub> H <sub>17</sub> N <sub>4</sub> OS	0.023	
C <sub>17</sub> H <sub>20</sub> N <sub>4</sub> O <sub>6</sub>	0.023	
C <sub>6</sub> H <sub>5</sub> NO <sub>2</sub>	0.023	
C <sub>18</sub> H <sub>32</sub> CaN <sub>2</sub> O <sub>10</sub>	0.023	
C <sub>8</sub> H <sub>11</sub> NO <sub>3</sub>	0.046	
C <sub>63</sub> H <sub>88</sub> CoN <sub>14</sub> O <sub>14</sub> P	0.0001	
C <sub>8</sub> H <sub>14</sub> O <sub>2</sub> S <sub>2</sub>	0.023	
NH <sub>2</sub> C <sub>6</sub> H <sub>4</sub> COOH	0.023	

removed by methanogenesis was calculated as the difference between total COD removed and (COD – S<sup>2-</sup>), corresponding to the quantity of COD removed via sulfidogenesis. These routes are the only way to direct the electron flux from the ethanol present in the synthetic wastewater.

$$\text{Total COD removal}(\text{COD}_{\text{Total}})(\%)$$

$$= \left( \frac{(\text{COD influent} - \text{COD effluent})}{\text{COD influent}} \right) \times 100 \quad (1)$$

$$\text{COD removal via sulfidogenesis } (\text{COD} - \text{S}^{2-})(\%)$$

$$= \left( \frac{0.67(\text{sulfate influent} - \text{sulfate effluent})}{(\text{COD influent} - \text{COD effluent})} \right) \times 100 \quad (2)$$

$$\text{COD removal via methanogenesis}(\text{COD} - \text{CH}_4)(\%)$$

$$= [(\text{COD}_{\text{Total}}) - (\text{COD} - \text{S}^{2-})](\%) \quad (3)$$

## RESULTS AND DISCUSSION

The organic matter was efficiently removed (>88%) through sulfate reduction via combined methanogenic and sulfidogenic pathways at a COD/sulfate ratio between 12.5 and 1.9 (Figures 2(a) and 2(b); Table 3). At higher COD/sulfate ratios, nearly 100% of the organic matter was removed. Under these higher COD/sulfate ratios, methanogenesis should prevail over sulfidogenesis (Dar *et al.* 2008).

According to Jeris & McCarty (1965), 70% of the methane produced by organic matter degradation derives from acetoclastic methanogenic fermentation, and 30% derives from H<sub>2</sub> respiration with CO<sub>2</sub> as the only electron acceptor. Therefore, up to 30% of the removed COD was derived from SRB incomplete oxidizers, as observed in stages 1, 2 and 3 (Table 3). In stages 4, 5 and 6, the sulfate reduction exceeded the maximum H<sub>2</sub> available, thus

**Table 2** | HAIS operating conditions

Compounds	Sulfidogenic and methanogenic evaluation stages					
	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6
COD (mg L <sup>-1</sup> )	1,500	1,500	1,500	1,500	1,500	1,500
Sulfate (mg L <sup>-1</sup> )	120	200	500	800	1,500	2,000
COD/sulfate ratio	12.5	7.5	3	1.9	1	0.75

**Table 3** | Statistical analysis of organic matter removal via sulfidogenesis and methanogenesis<sup>a</sup>

	Stages					
	1	2	3	4	5	6
COD/sulfate	12.5	7.5	3	1.9	1.0	0.75
Total COD removal (%)	97.1 ± 2.4	95.0 ± 1.6	93.8 ± 3.1	87.9 ± 3.7	81.2 ± 3.3	86.5 ± 6.9
COD removal via sulfidogenesis (%)	1.8 ± 2.0	4.7 ± 2.6	20.3 ± 5.0	32.8 ± 2.3	44.7 ± 6.3	42.7 ± 5.2
COD removal via methanogenesis (%)	94.6 ± 4.2	91.4 ± 4.0	74.7 ± 6.9	54.1 ± 6.0	36.6 ± 6.3	44.1 ± 5.4
N (number of data)	08	07	14	07	07	09

<sup>a</sup>According to Equations (1)–(3).

confirming the consumption of acetate by SRB complete oxidizers. Figure 4 corroborates this hypothesis. The removal of organic matter was greater than that predicted by the sulfate reduction stoichiometric equation (Figure 2), indicating the coexistence of sulfidogenic and methanogenic pathways.

In stage 5, the biological sulfate reduction efficiency decreased from that of earlier stages, with nearly 40% of the influent sulfate concentration remaining (Figure 2(b)). However, the quantity of sulfate removed increased (COD/sulfate ratio of 1), but this removal was not proportional to the quantity of sulfate loaded (Figure 2(b)), which indicated limitations on the electron availability for sulfidogenesis. According to Damianovic *et al.* (2006), incomplete oxidation

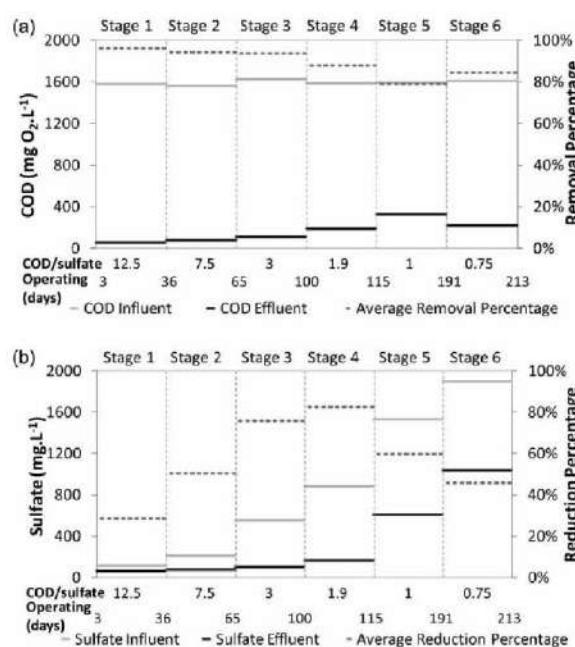
of ethanol by SRB requires a COD/sulfate ratio of approximately 1.92 for efficient sulfidogenesis.

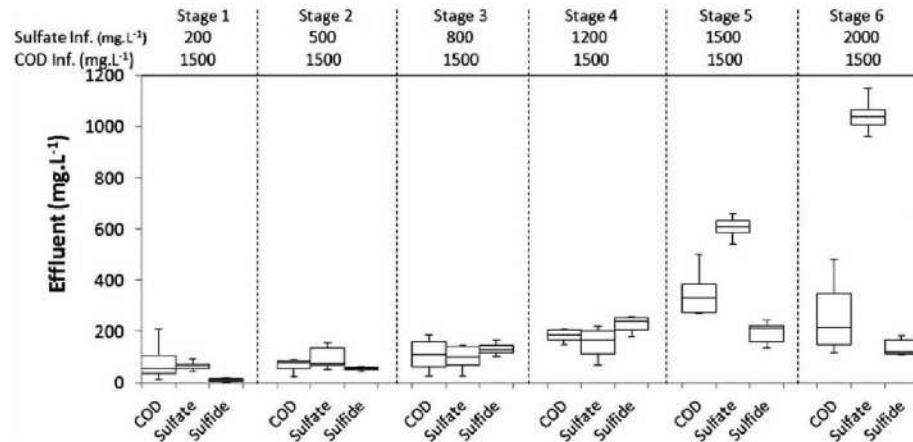
The same result occurred in stage 6 (Figure 2(b)), with a similar quantity of sulfate reduced as in the previous stage, confirming the hypothesized limited electron availability. Regarding the COD, both the removal efficiency and the quantity removed decreased from stage 3 onward. At stage 4, the COD removal efficiency was approximately 90% and decreased to 80% in stage 5 (Figure 2(a)) when the sulfide concentration reached 250 mg L<sup>-1</sup>. A slight increase in the COD removal efficiency in stage 6 (Figure 2(a)) coincided with the reduction of sulfide (Figure 2(b)) to 150 mg L<sup>-1</sup>. The data obtained do not provide a clear explanation for the decreased sulfide concentration in the liquid. Furthermore, the sulfide concentrations were below the expected stoichiometric values, suggesting the formation of compounds of intermediate oxidation state such as sulfite and/or thiosulfate (Dannenberg *et al.* 1992). However, the acclimation of methanogenic organisms to sulfide (Muyzer & Stams 2008; Silva *et al.* 2011) can explain the increased organic matter removal observed in stage 6 (Figure 2(a)).

Figure 3 presents a statistical analysis of the COD, sulfate and sulfide data throughout the experiment.

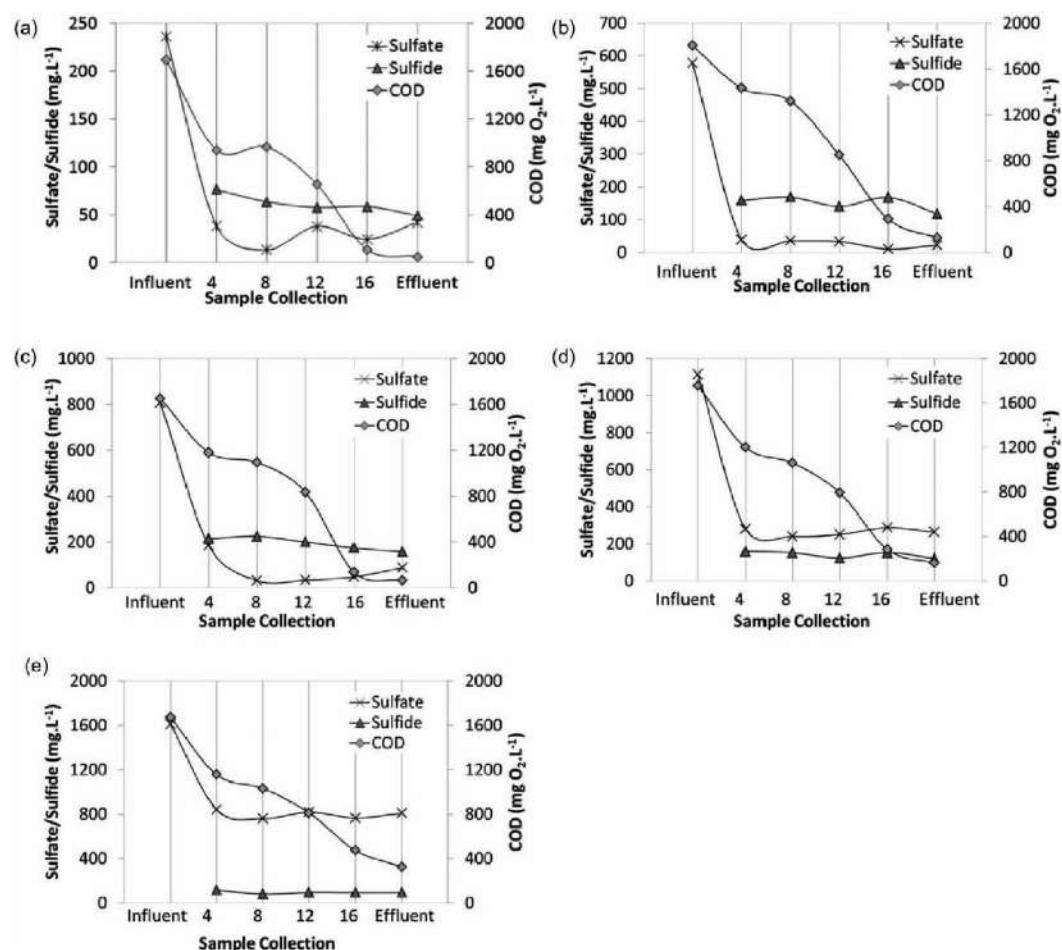
The sulfate removal was unstable during stages 1 and 2 (~40%) at COD/sulfate ratios of 12.5 and 7.5, respectively (Figure 3). Sulfur is an essential nutrient for methanogens (Speece 1983). Therefore, at low sulfide concentrations, such as those in stages 1 and 2 (Figure 3), the sulfide likely stimulated methane formation (Table 2), as observed in the UASB reactor experiments by Paula & Foresti (2009). As expected, the increased sulfide concentration caused a gradual increase in the effluent COD, likely due to competition for electron donors.

Despite the slight increase in the average COD removal efficiency during stage 6, the overall organic matter removal was quite unstable, unlike the stable sulfidogenesis

**Figure 2** | (a) Average quantity of COD removed; (b) average quantity of sulfate reduction.



**Figure 3** | Boxplot analysis of organic matter removal (COD), sulfate reduction and sulfide concentration.



**Figure 4** | Reactor concentration gradients: (a) stage 2 – sulfate concentration = 200 mg L<sup>-1</sup>; (b) stage 3 – sulfate concentration = 500 mg L<sup>-1</sup>; (c) stage 4 – sulfate concentration = 800 mg L<sup>-1</sup>; (d) stage 5 – sulfate concentration = 1,500 mg L<sup>-1</sup>; (e) stage 6 – sulfate concentration = 2,000 mg L<sup>-1</sup>.

(Figure 3). With limited availability of electron donors, the competing roles of the incomplete and complete oxidizing SRB remain uncertain.

The highest average sulfate reduction efficiency and stability occurred during stage 4 (Table 3; Figure 3). A previous study using an HAIS reactor detected acetogenesis associated with sulfate reduction followed by methanogenesis (Damianovic & Foresti 2009). Sulfate reduction and organic matter removal occurred as sequential reactions along the HAIS reactor. The same phenomenon was observed in this study (Figure 3).

The concentration gradients of the COD, sulfate and sulfide indicated that the sulfidogenic activity occurred at a higher rate in the first section of the reactor, from the influent inlet to the first sampling port, than in the other sections – for all stages. Organic matter was removed along the entire length of the reactor, as indicated by the COD profiles.

The high sulfate reduction efficiencies obtained at the first sampling port (Figure 4) can be attributed to the intense SRB activity, whereas the COD removal via methanogenesis prevails from port 8 onward. From ports 4 to 8, the COD was level, indicating a transition zone, possibly responsible for the acclimation of MA to sulfide.

## CONCLUSIONS

The operation of the HAIS reactor at decreasing COD/sulfate ratios provided a better understanding of the sulfidogenesis and methanogenesis processes that occur along a non-ideal plug-flow unit containing immobilized biomass.

At a high COD/sulfate ratio (12.5 and 7.5), the organic matter removal was stable and efficient, primarily due to methanogenesis. The highest sulfate reduction efficiency was obtained at a COD/sulfate ratio of 3 to 1.9 for influent sulfate concentrations up to  $800 \text{ mg L}^{-1}$ . The increase in the influent sulfate concentration for COD/sulfate ratios of 1 and 0.75 did not increase the quantity of sulfate reduced owing to limited electron donor availability. However, the overall COD removal efficiency decreased. The quantities of sulfate reduced at ratios of 1 and 0.75 were quite similar. The highest COD and sulfate removal rates were not achieved at the same COD/sulfate ratio; thus, competition was the primary mechanism in establishing the electron flux.

The high sulfate reduction in the first reactor segment led to a gradual COD removal via methanogenesis along the subsequent segments. The increase in sulfide

concentration with decreasing COD/sulfate ratio resulted in a decrease in the COD removal rate along the reactor. At a COD/sulfate ratio of 0.75, sulfidogenesis was likely inhibited by the competition for electron sources.

## ACKNOWLEDGEMENTS

The research grants for this work were funded by the Brazilian agencies FAPESP (São Paulo Research Foundation) and CNPq (National Council for Scientific and Technological Development).

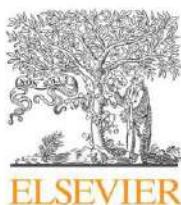
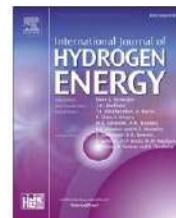
## REFERENCES

- APHA 2005 *Standard Methods for Examination of Water and Wastewater* 19th edn, American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, USA.
- Bryant, M. P., Wolin, E. A., Wolin, M. J. & Wolfe, R. S. 1967 *Methanobacillus omelianskii* a symbiotic association of two species of bacteria. *Archiv für Microbiology* **59**, 20–31.
- Cao, J., Zhang, G., Mao, Z., Li, Y., Fang, F. & Chao, Y. 2012 Influence of electron donors on the growth and activity of sulphate-reducing bacteria. *International Journal of Mineral Processing* **106–109**, 58–64.
- Colleran, E., Finnegan, S. & Lens, P. 1995 Anaerobic treatment of sulphate-containing waste streams. *Antonie van Leeuwenhoek* **67**, 29–46.
- Damianovic, M. H. R. Z. & Foresti, E. 2009 Dynamics of sulfetogenesis associated to methanogenesis in horizontal-flow anaerobic immobilized biomass reactor. *Process Biochemistry* **44**, 1050–1054.
- Damianovic, M. H. R. Z., Sakamoto, I. K. & Foresti, E. 2006 Biofilm adaptation to sulfate reduction in anaerobic immobilized biomass reactors subjected to different COD/sulphate ratios. *Water Science and Technology* **54** (2), 119–126.
- Dannenberg, S., Kirder, M., Dilling, W. & Cypionka, H. 1992 Oxidation of  $\text{H}_2$ , organic compounds and inorganic sulfur compounds coupled reduction of  $\text{O}_2$  or nitrate by sulfate reducing bacteria. *Archives of Microbiology* **158**, 93–99.
- Dar, S. A., Kleerebezem, R., Stams, A. J. M., Kuenen, J. G. & Muyzer, G. 2008 Competition and coexistence of sulphate-reducing bacteria, acetogens and methanogens in a lab-scale anaerobic bioreactor as affected by changing substrate to sulphate ratio. *Applied Microbiology Biotechnology* **78**, 1045–1055.
- Foresti, E., Zaiat, M., Cabral, A. K. A. & Del Nery, V. 1995 Horizontal-flow anaerobic immobilized sludge (HAIS) reactor for paper industry wastewater treatment. *Brazilian Journal of Chemical Engineering* **12** (4), 235–239.

- Hulshoff Pol, L. W., Lens, P. N. L., Stams, A. J. M. & Lettinga, G. 1998 Anaerobic treatment of sulphate-rich wastewaters. *Biodegradation* **9** (3–4), 213–224.
- Jeris, J. S. & McCarty, P. L. J. 1965 The biochemistry of methane fermentation using C<sup>14</sup> tracers. *Water Pollution Control Federation* **57**, 178–192.
- Lens, P. N. L., Visser, A., Janssen, A. J. H., Hulshoff Pol, L. W. & Lettinga, G. 1998 Biotechnological treatment of sulfate-rich wastewaters. *Critical Reviews in Environmental Science and Technology* **1** (28), 41–88.
- Li, L., Wang, J., Luan, Z., Ji, Z. & Yu, L. 2012 Biological sulphate removal from acrylic fiber manufacturing wastewater using a two-stage UASB reactor. *Journal of Environmental Sciences* **24** (2), 343–350.
- Mulopo, J., Greben, H., Sigama, J., Radebe, V., Mashego, M. & Burke, L. 2011 The relationships between sulphate reduction and COD/VFA utilization using grass cellulose as carbon and energy sources. *Applied Biochemistry Biotechnology* **163**, 393–403.
- Muyzer, G. & Stams, A. J. M. 2008 The ecology and biotechnology of sulfate-reducing bacteria. *Nature Reviews: Microbiology* **6**, 441–454.
- Omil, F., Lens, P. N. L., Visser, A., Hulshoff Pol, L. W. & Lettinga, G. 1998 Long-term competition between sulfate reducing and methanogenic bacteria in UASB reactors treating volatile fatty acids. *Biotechnology Bioengineering* **57**, 676–685.
- Paula Jr, D. & Foresti, E. 2009 Sulfide toxicity kinetics of a UASB reactor. *Brazilian Journal of Chemical Engineering* **26** (4), 669–675.
- Silva, A. J., Domingues, M. R., Hirasawa, J. S., Varesche, M. B. A., Foresti, E. & Zaiat, M. 2011 Kinetic modeling and microbial assessment by fluorescent in situ hybridization in anaerobic sequencing batch biofilm reactors treating sulphate-rich wastewater. *Brazilian Journal of Chemical Engineering* **28** (2), 209–219.
- Speece, E. R. 1983 Anaerobic biotechnology for industrial wastewater treatment. *Environmental Science & Technology* **17** (9), 416A–427A.
- Subtil, E. L., Cassini, S. T. A. & Gonçalves, R. F. 2011 Sulphate and dissolved sulfide variation under low COD/sulphate ratio in up-flow anaerobic sludge blanket (UASB) treating domestic wastewater. *Interdisciplinary Journal of Applied Science* **7** (1), 130–139.
- Touzel, J. P. & Albagnac, G. 1983 Isolation and characterization of *Methanococcus mazei* strain MC<sub>3</sub>. *FEMS Microbiology Letters* **16** (2–3), 241–245.
- Visser, A., Gao, Y. & Lettinga, G. 1993 Effects of pH on methanogenesis and sulphate reduction in thermophilic (55°C) UASB reactors. *Bioresource Technology* **44**, 113–121.
- Zehnder, A. J. B. & Wuhrmann, K. 1977 Physiology of a *Methanobacterium* strain AZ. *Archives of Microbiology* **111** (3), 199–205.

First received 16 July 2013; accepted in revised form 27 January 2014. Available online 8 February 2014

INTERNATIONAL JOURNAL OF HYDROGEN ENERGY 44 (2019) 27259–27271

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)**ScienceDirect**journal homepage: [www.elsevier.com/locate/he](http://www.elsevier.com/locate/he)

## Hydrogen production in reactors: The influence of organic loading rate, inoculum and support material



Rogério Vilela <sup>a</sup>, Flávia Talarico Saia <sup>b</sup>, Gustavo Bueno Gregoracci <sup>b</sup>,  
 Rubens Duarte <sup>c</sup>, Pedro Andrade <sup>d</sup>, Bas van der Zaan <sup>e</sup>, Alette Langenhoff <sup>f</sup>,  
 Márcia H.R. Z. Damianovic <sup>a,\*</sup>

<sup>a</sup> Biological Process Laboratory, São Carlos School of Engineering, University of São Paulo, Environmental Engineering, Bloco 4-F, Av. João Dagnone, 1100, Santa Angelina, 13563-120, São Carlos, SP, Brazil

<sup>b</sup> Marine Institute, Federal University of São Paulo, Rua Dr. Carvalho de Mendonça 144, 11070-102, Santos, SP, Brazil

<sup>c</sup> Center of Biological Sciences, Federal University of Santa Catarina, Campus Reitor João David Ferreira Lima, Bairro Trindade, 88040-900, Florianópolis, SC, Brazil

<sup>d</sup> Department of Soil Science, "Luiz de Queiroz" College of Agriculture, University of São Paulo, 13418-900, Piracicaba, SP, Brazil

<sup>e</sup> Deltares, Daltonlaan 600, 3584 BK Utrecht, the Netherlands

<sup>f</sup> Department of Environmental Technology, Wageningen University and Research, P.O. Box 17, 6700 AA, Wageningen, the Netherlands

### HIGHLIGHTS

- Sugarcane molasses was suitable for thermophilic hydrogen production.
- Inocula, support material and OLR influenced hydrogen production.
- Thermoanaerobacterium, Clostridium sensu stricto and Thermotoga were selected.

### ARTICLE INFO

#### Article history:

Received 30 June 2019

Received in revised form

20 August 2019

Accepted 22 August 2019

Available online 13 September 2019

### ABSTRACT

Hydrogen production was evaluated in two thermophilic structured bed (USBR) reactors. USBR1 was inoculated with auto-fermented sugarcane vinasse and low-density polyethylene cubes were used as support material. USBR2 was inoculated with anaerobic sludge from an up-flow anaerobic sludge blanket (UASB) reactor treating sugarcane vinasse, and polyurethane foam matrices was used as support material. The reactors were operated in parallel with sugar cane molasses at organic loading rate (OLR) from 30 to 120 g COD L<sup>-1</sup>d<sup>-1</sup> during 45 days. Hydrogen production was detected during the whole operational period, with maximum values of 1123 mL H<sub>2</sub> d<sup>-1</sup>L<sup>-1</sup> and 2041 mL H<sub>2</sub> d<sup>-1</sup>L<sup>-1</sup> for USBR1 and USBR2, respectively. The number of gene copies encoding for Fe-hydrogenase was higher in USBR2 for all OLR applied. DNA sequences related to Thermoanaerobacterium and Clostridium sensu stricto were predominant in USBR1. In USBR2, in addition to these microorganisms, *Lactobacillus*, *Pseudomonas* and *Thermotoga*, and sequences with low frequency of abundance (<5%) involved directly and indirectly in hydrogen production were also present. The taxonomical and functional more diverse inoculum of USBR2 was

\* Corresponding author.

E-mail address: [ftsaia@yahoo.com.br](mailto:ftsaia@yahoo.com.br) (M.H.R.Z. Damianovic).

<https://doi.org/10.1016/j.ijhydene.2019.08.180>

0360-3199/© 2019 Hydrogen Energy Publications LLC. Published by Elsevier Ltd. All rights reserved.

associated with a higher hydrogen production. Besides fermentation, an unknown metabolism was relevant in USBR2, revealing the importance of physiological characterization of the microbial community present.

© 2019 Hydrogen Energy Publications LLC. Published by Elsevier Ltd. All rights reserved.

## Introduction

Brazil is the World's largest sugar and ethanol producer from sugarcane crops. The São Paulo state is responsible for respectively 55% and 44% of ethanol and sugar production. Per ton of sugarcane, 33 L of ethanol and 67 kg of sugar are processed [1]. Sugarcane molasses (SM) is an intermediate of ethanol production and is produced in high amounts, approximately 40–60 kg/ton of sugarcane processed. It possesses relatively high organic matter content, with approximately 60% of dissolved solids composed by sucrose, glucose and fructose [2] plus nutrient minerals. Due to its high organic content and nutrient minerals, SM can be converted by dark fermentation processes into hydrogen, ethanol and volatile fatty acids (VFAs), however, there are few reports on the utilization of molasses as a sole carbon source for hydrogen production [3–5]. Hydrogen is a sustainable energy carrier which is clean, efficient (with high-energy yield, 122 kJ/g) and renewable, and it does not generate any toxic byproducts [6,7]. It can be generated biologically by photosynthetic microbes trough photosynthetic process or by acidogenic microorganisms trough dark fermentation. Dark fermentation has advantageous over photosynthetic process: light is not necessary, no oxygen is present in the system to inhibit the activity of hydrogenases, and a wide variety of feedstocks can be used, including SM and wastewater containing high content of carbohydrates commonly discharged in agricultural processing [8]. Among the factors to select and maintain active microorganisms capable of continuous hydrogen production, literature have shown that configuration of reactor, hydraulic retention time, source of inocula, support material and temperature are important [3–8].

Different configurations of anaerobic reactors using suspended and fixed microorganisms have been studied for hydrogen production. When using low retention times, suspended microorganisms are easily washed out of the reactor, while the accumulation of biomass occurs in fixed bed reactors. Recently, a new configuration of reactor was developed based on the use of a fixed and structured bed as an immobilization matrix for anaerobic biomass [8]. This configuration combines the advantages of immobilized cell growth, such as lower sensitivity to environmental variations (i.e., pH, temperature and organic loading rate [OLR]) and higher substrate conversion rates, with higher bed porosity, preventing the accumulation of extracellular polymeric compounds and suspended solids [7,8]. The presence of support material for microbial biofilm formation is an important factor in enriching microorganisms in bed reactors, which is crucial for the successful implementation and performance of these reactors [11,12]. So far, different

support materials have been evaluated for hydrogen production [9–12], but there is limited understanding how support materials influence hydrogen production using a structured bed reactor [13,14].

In addition to the reactor type, the applied OLR influences the hydrogen production by fermentative process. The OLR can be varied by changes in hydraulic retention time [9,14] or substrate concentration [11,14], showing in both cases shifts in microbial community composition and overall performance of the reactors. Literature also reports that the source of inocula can affect hydrogen production [15,16], but studies related to structured bed reactor linking microorganisms to hydrogen production are missing.

Another parameter for hydrogen production performance is the temperature. Thermophilic fermentation can provide higher rates and yields of hydrogen production than mesophilic conditions. A thermophilic temperature decreases the inhibition of hydrogen production that is caused by high hydrogen partial pressure in the liquid phase [17,18]. Continuous hydrogen production in high rate and yield is important for application of the technology, and there are scarce studies employing sugarcane molasses [19,20].

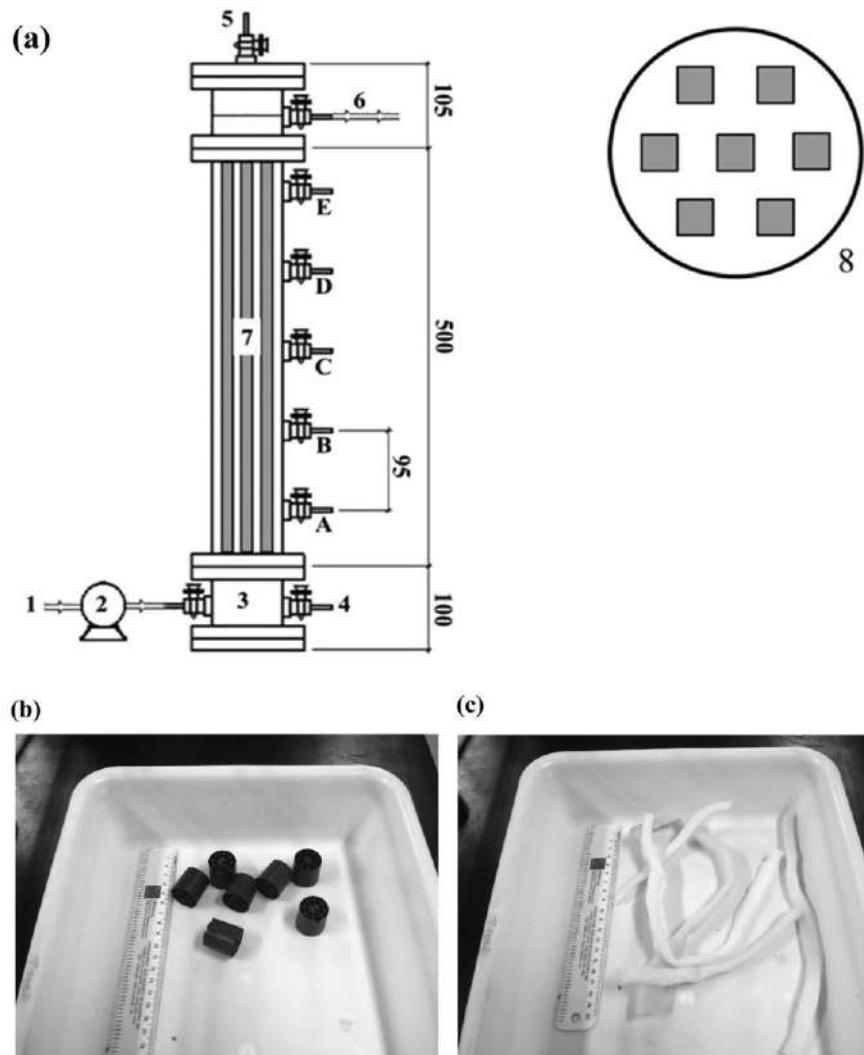
In this study, we varied the organic matter concentration (COD) to evaluate the effect of the OLR on thermophilic hydrogen production using up-flow anaerobic fixed-structured bed reactors (USBR) fed continuously with sugarcane molasses. The effect of the operational condition on the microbial communities that developed in the reactors was studied using 16S rRNA gene metagenomics and the number of genes encoding for Fe-hydrogenase was quantified by real-time PCR.

## Material and methods

### Reactors, inocula and support material

Two anaerobic up-flow structured bed reactors (USBR) [8] were used in the experiments. The USBR reactor, with bed structured longitudinally, allows for biomass control, minimizing bed clogging. The reactors were made of acrylic tubes, filled with different support materials and different inocula. The total and liquid volumes of the reactors were 2.2 L and 1.65 L, respectively. Fig. 1 shows a scheme of the reactor and support materials used.

In reactor USBR1, low-density polyethylene cylinders were used as support material (Fig. 1b; Table 1). The bed was structured with five cylinders organized in thirteen stainless steel rod columns. The bed porosity was 76%. Inoculation was performed with auto-fermented sugarcane vinasse. The



**Fig. 1 – (a)** Anaerobic reactor with structured bed: 1- Influent inlet, 2-peristaltic pump, 3- mixture zone, 4- head zone, 5- headspace, 6-headspace, 7-effluent outlet, 8-cross-sectional view of the bed (structured fixed bed); **(b)** Low density polyethylene cylinders; **(c)** polyurethane foam matrices.

**Table 1 – Characteristics of the support materials used in the experiment.**

Support material	Shape	Length (mm)	Diameter/edge (mm)	Specific surface area ( $\text{cm}^2 \cdot \text{g}^{-1}$ )	Density $\text{g} \cdot \text{L}^{-1}$
Low-density polyethylene	Cylinder	40	13	9.4	0.960
Polyurethane foam	Prismatic	600	10	25.5	0.035

inoculum was obtained through the natural sugarcane vinasse fermentation process. Vinasse was filtered with a paper filter (Nalgene, density of  $80 \text{ g m}^{-2}$ , porosity of  $3 \mu\text{m}$ ) to reduce the concentration of suspended solids. The filtered vinasse with an adjusted pH of 6.5 was maintained for three consecutive days in a dark chamber to allow for autochthonous microorganism growth. After this period, the fermented effluent with the cultured biomass was pumped into the reactor and recirculated for five consecutive days to enhance the attachment of the biomass to the support material, as previously described in [11].

In reactor USBR2 polyurethane foam matrices were used as support material (Fig. 1c). The support material was laid out in nine vertical strips of prismatic polyurethane foam which were attached to the reactor extremities. The bed porosity was 73%. Inoculation was performed with anaerobic sludge from an up-flow anaerobic sludge blanket (UASB) reactor treating vinasse wastewater under thermophilic conditions and inoculation was performed as follows. Granulated sludge was macerated and kept in contact with the support material for 24 h. Then, the reactor was kept under effluent recirculation for five consecutive days to enhance the attachment of the

biomass to the support material, as previously described in [21].

#### Operation of the reactors

USBR1 and USBR2 were operated at 55 °C and fed continuously with sugar cane molasses for 45 days with a hydraulic retention time (HRT) of 2 h, which corresponds to a flow rate of 19.8 L<sup>-1</sup> d<sup>-1</sup>. The reactors were fed with a peristaltic pump and the feeding flask was kept at 4 °C. Organic loading rates (OLRs) values were approximately: 30; 60 and 120 g COD.L<sup>-1</sup>.d<sup>-1</sup>, corresponding to concentrations of 2.5; 5 and 10 g COD.L<sup>-1</sup>, respectively. The reactors were operated from day 1 to day 12 with an OLR of 30 g COD.L<sup>-1</sup>.d<sup>-1</sup>; from day 12 to day 26 with an OLR of 60 g COD.L<sup>-1</sup>.d<sup>-1</sup> and from day 26 to day 45 with an OLR of 120g COD.L<sup>-1</sup>.d<sup>-1</sup>.

#### Sugarcane molasses

Sugarcane molasses (SM) was collected at the São Martinho Plant (Pradópolis, SP, Brazil) and stored at 4 °C. It was diluted with tap water to obtain organic loads of 30, 60 and 120 g COD.L<sup>-1</sup>.d<sup>-1</sup>. The reactors were operated with natural pH of the sugarcane molasses. The chemical composition of diluted SM (10g of SM/1L of distilled water) is the following (all in mg.L<sup>-1</sup>): Total COD (8,720) soluble COD (0.45 µm) (7,880); BOD (4,000); total nitrogen (270); sulfate (70); sulfite (0.2); fenol (0.1); glucose (2,440) and fructose (845); total solids (7,651); fixed total solids (520); volatile total solids (7,130); soluble total solids (7,450); fixed soluble solids (50); volatile soluble solids (6,960); cadmium (0.05); lead (0.06); calcium (287); copper (0.05); total chrome (0.04); iron (0.8); magnesium (21); manganese (0.3); nickel (0.6); potassium (216); sodium (4.7) and zinc (0.17).

#### Chemical analyses

To evaluate the performance of the reactors, samples were taken of the influent and effluent, three times per week and analysed in triplicate for COD, pH [22], total carbohydrates [23], and total acids [24]. Organic acids (acetic, propionic, lactic, isobutyric, butyric, citric, malic, succinic, formic, valeric, isovaleric and caproic) were analyzed with a high-performance liquid chromatograph (HPLC) [15]. The volume of produced biogas was measured by Milligas Counter - Ritter®. The composition of biogas, including hydrogen, methane and carbon dioxide, was analyzed using a gas chromatograph Shimadzu GC-2010 [15].

The hydrogen production rate (HPR) and volumetric hydrogen production (VHP) were calculated at standard temperature and pressure for OLRs of 60 and 120 g COD.L<sup>-1</sup>.d<sup>-1</sup> according to equations (1) and (2).

$$\text{HPR} = Q_b \times \% \text{ H}_2 \quad \text{Eq. 1}$$

$$\text{VHP} = \frac{Q_b \times \% \text{ H}_2}{V_{\text{liquid}}} \quad \text{Eq. 2}$$

Where Q<sub>b</sub> is flow rate (L.d<sup>-1</sup>) of hydrogen produced; %H<sub>2</sub> is percentage of hydrogen in the biogas; V<sub>liquid</sub> is the liquid volume of the reactor.

#### Molecular analyses

##### DNA extraction

Biomass attached to polyurethane foams and low-density polyethylene (LDP) was sampled at the top of the reactors at the end of each operational phase. Due to the configuration of the reactors it was not possible to sample support material along the reactor. Suspended cells were sampled from the sampling points along the length of the reactors at the end of each operational phase. Biomass was removed manually from the support materials by successive washing in PBS-buffer (PBS: 0.13 M NaCl, 7 mM Na<sub>2</sub>HPO<sub>4</sub>, 3 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 7.2) and subsequent centrifugation for 10 min at 6000 rpm, at 4 °C. Afterwards, the pellet (~1 g) was collected and kept at -20 °C until DNA extraction. Biofilm and suspended samples were pooled in a composed sample for each operational phase. For metagenomic analysis, genomic DNA was extracted using a phenol:chloroform protocol previously described in [25]. For qPCR analysis, genomic DNA was extracted using PowerSoil® DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA), according to the manufacturer's protocol. DNA quality was assessed by an ND-2000 spectrophotometer (Nanodrop Inc., Wilmington, DE), using a ratio of 260/280 nm > 1.8, and agarose gel electrophoresis.

##### Metagenomic and qPCR analyses

For metagenomic analysis, the 16S rRNA genes were amplified using the primer set S-D-Bact-0341-b-S-17 (5'-CCTACGGGNNGCWGCAG-3') and S-D-Bact-0785-a-A-21 (5'-GACTACHVGGGTATCTAATCC-3'), flanking the V3 and V4 hypervariable regions [26]. Amplicon sequencing was performed at the Functional Genomics Center (ESALQ/USP Piracicaba, Brazil) on an Illumina MiSeq platform, following the manufacturer's guidelines.

Short reads (less than 50bp) were removed, and raw reads were dynamically trimmed using SolexaQA++ [27], using standard parameters. Paired-ends were reorganized for joining with PAIRFQ (open licence) and joined with PANDASEQ [28]. Chimeric sequences were checked with Uchime [29], and exact replicates were joined with grep in command line. SWARM was used to cluster OTUs, using default configuration [30]. Finally, Mothur 1.36 [31] was used for annotation (classify.seqs) against Silva SSU (v1.28) [32], properly formatted and made available by Mothur. After annotation, we attempted to infer metabolic functions using the database provided by [33]. Statistical comparison was performed using STAMP [34].

Genes encoding for Fe-hydrogenase were quantified by qPCR. DNA samples were amplified with the set of primers Hyda-F (5'TCACCAACAAATTTGGT-3') and HydA-R (5'GCTGCTTCATAACTCC-3') [35] following the procedures previously described [35]. The standards were determined using an 8-fold dilution of a previous amplification of the genes. For further use of the PCR product, they were purified using a Charge Switch PCR clean up (Invitrogen, USA). Therefore, the calibration curves were calculated with four replicates, and the efficiency obtained was 91%. The amplification reactions were performed in a StepOne™ System Applied Biosystems (Thermofisher, USA). The reaction was

performed in triplicate, and the average and standard deviation were determined. DNA was quantified using a fluorometer (Qubit 2.0, Invitrogen, Carlsbad, CA, USA). The results were expressed in copies of Fe-hydrogenase/ng DNA.

## Results and discussion

### Performance of the bioreactors

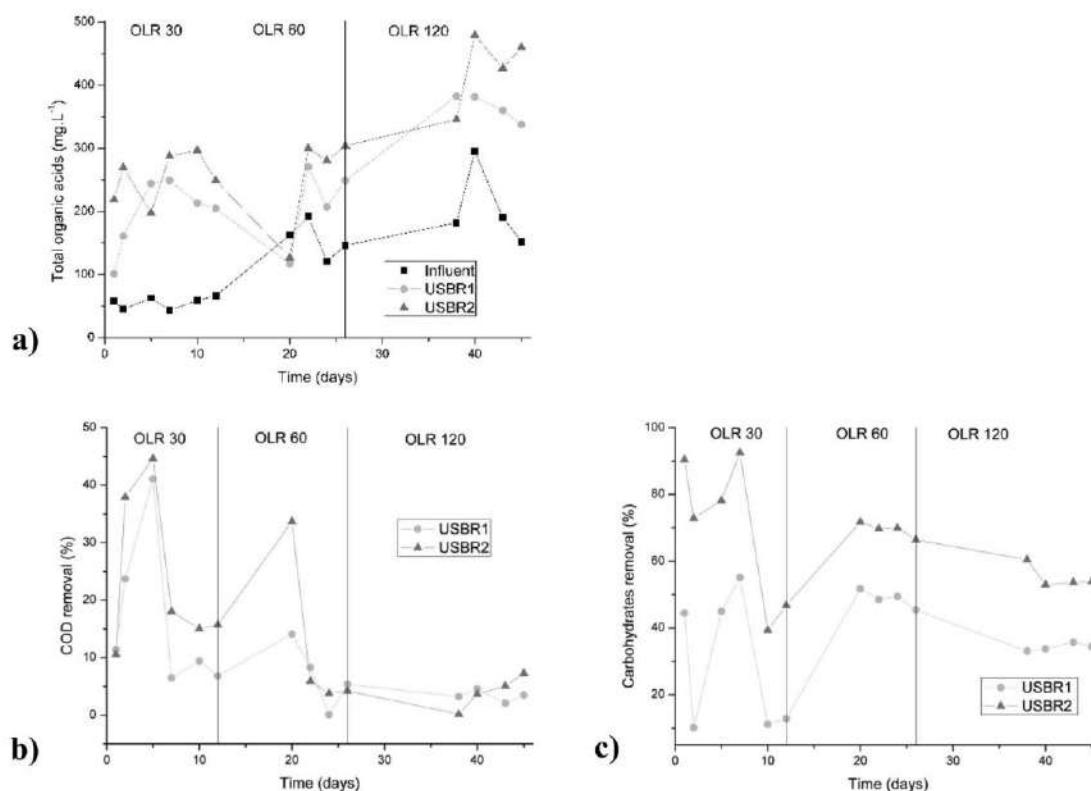
Two lab-scale up-flow structured bed reactors were operated under thermophilic condition (55 °C) for 45 days at three different organic loading rates conditions. No additions were added to the reactor influent to control the pH, which ranged from 4.5 to 6.5. Effluent pH values were stable during all operational period for both reactors with mean values of  $4.1 \pm 0.2$  (USBR1) and  $4.3 \pm 0.2$  (USBR2) (Table 1S – supplement material) and the production of acids (Fig. 2a; Table 1S – supplement material), implying acidogenesis. In this study, hydrogen production was observed at pH 4.1 (USBR1) and 4.3 (USBR2), which is in line with literature studies that report pH values ranging from 4 to 5.5 and shows that optimal pH scopes for hydrogen production may be different due to microbial communities under different operational conditions. The no need to control pH value is important for application of anaerobic technology, since save costs with addition of alkalis [36–38].

COD removal was higher in USBR2 than in USBR1. For both reactors, removal of COD was higher at the lowest OLR,

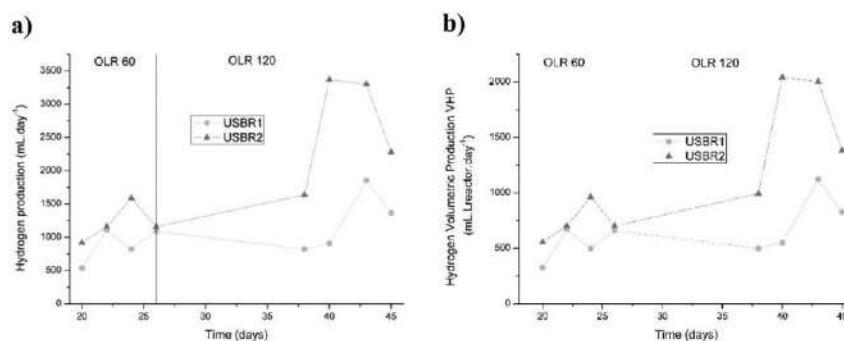
decreasing with the increase of OLR (Fig. 2b). The organic matter conversion resulted in acetic and butyric acids and hydrogen production (Fig. 4). Carbohydrates conversion was also higher in USBR2 than USBR1, with average values of 30%, 50% and 35% in USBR1 and 70%, 69% and 55% in USBR2, with OLR applied of 30, 60 and 120 g COD.L<sup>-1</sup>.d<sup>-1</sup>, respectively (Fig. 2c). Table 1S (supplement material) shows the maximum and average values of parameters monitored. The average values of COD and carbohydrates removal were similar to the ones observed in thermophilic and mesophilic systems fed with molasses [3,19,36], and thermophilic systems fed with cheese whey [37], sugarcane vinasse [11,18] and synthetic wastewater containing glucose [38] and sucrose [39].

### Hydrogen production

Continuous hydrogen production was observed during the operational period of 45 days, with higher values in USBR2 than in USBR1. The average hydrogen content in the biogas of USBR1 was 48%, 58% and 56%, for OLRs of 30, 60 and 120 g COD.L<sup>-1</sup>.d<sup>-1</sup> respectively. In USBR2, the biogas had an average hydrogen content of 57%, 58% and 54%, for OLRs of 30, 60 and 120 g COD.L<sup>-1</sup>.d<sup>-1</sup> respectively. Methane was not detected in the biogas during the operational period while hydrogen was continuously produced (Fig. 3). These results show that the operational conditions applied in our study with hydraulic retention time (HRT) of 2 h, pH 4.3 and a thermophilic temperature could inhibit the activity of hydrogen consumers, such as homoacetogens and hydrogenotrophic methanogens.



**Fig. 2 – Performance of reactors USBR1 and USBR2 operated with different organic loading rates. (a) total organic acids; (b) total organic matter (COD) removal; (d) carbohydrates removal. OLR: g COD.L<sup>-1</sup>.d<sup>-1</sup>.**



**Fig. 3 – (a) Hydrogen production rate (HPR) and (b) volumetric hydrogen production (VHP) during the experimental period of reactors USBR1 and USBR2 operated with different organic loading rate (OLR). OLR: g COD.L<sup>-1</sup>.d<sup>-1</sup>.**

Low frequencies (<5%) of sequences relating to *Methanothermobacter* were detected in USBR2 at 120 g COD.L<sup>-1</sup>.d<sup>-1</sup> (Fig. 7), no contributing for methane detection. Some authors show that pretreatment of inocula using mixed cultures enhanced hydrogen production by selecting hydrogen producing bacteria and suppressing hydrogen consumers [11,16]. Our findings show that pretreatment of the inocula was not needed, which gives an important cost saving aspect in the application of the technology. Similar results were also observed by others [3,11,38].

According to Fig. 3, the hydrogen production rate (HPR) and volumetric hydrogen production (VHP) were higher in USBR2 than in USBR1, and for USBR2 the values increased with OLR. These results are in accordance with quantification of the genes encoding for Fe-hydrogenase. Table 2 shows that the number of copies of this gene was also higher in USBR2 than in USBR1, like the HPR and VHP. In addition, the number copies of genes encoding for Fe-hydrogenase in USBR1 had low variation when the OLR increased, while the number of genes encoding for Fe-hydrogenase in USBR2 increased (mainly

from 30 to 60 g COD.L<sup>-1</sup>.d<sup>-1</sup>). The number of genes encoding for Fe-hydrogenase present over time confirm that continuous hydrogen production from sugarcane molasses was maintained at thermophilic conditions, as also observed by Ferraz Jr et al. [18].

We obtained a maximum volumetric production of hydrogen (VHP) of 1123 mL H<sub>2</sub>.L<sup>-1</sup>.d<sup>-1</sup> (USBR1) and 2041 mL H<sub>2</sub>.L<sup>-1</sup>.d<sup>-1</sup> (USBR2), which was lower than most other anaerobic systems fed with subproducts of sugarcane industry (Table 3) and operated under thermophilic condition, except for the values obtained by Ferraz Jr et al. [18], when comparing with the USBR2. For USBR1 the values are similar, since the reactor was operated with a similar substrate, under thermophilic condition using the same support material (low density polyethylene) and the same inoculum (autofermented vinasse). Interesting to note that for mesophilic condition, the volumetric hydrogen production obtained by Ferraz Jr et al. [11] was lower than obtained under thermophilic condition [18], showing that, OLR and temperature influenced on hydrogen production. The hydrogen yields obtained in our

**Table 2 – Gene copy numbers for Fe-hydrogenase in DNA samples taken from the reactors USBR1 and USBR2 with different OLR of 30, 60 and 120 g COD.L<sup>-1</sup>.d<sup>-1</sup> at days 12, 26 and 45 of operation.**

Time (days)	OLR g COD.L <sup>-1</sup> .d <sup>-1</sup>	Log n. copies of genes encoding for Fe-hydrogenase.ng <sup>-1</sup> DNA	
		USBR1	USBR2
12	30	6.7	7.6
26	60	6.4	7.8
45	120	6.9	10

**Table 3 – Overview of maximum values of volumetric hydrogen (VHP) production and hydrogen yield from dark fermentative hydrogen production systems fed with subproducts of sugarcane industry.**

Reactor and temperature	Substrate	HRT Hr	OLR applied gCOD.L <sup>-1</sup> .d <sup>-1</sup>	Maximum VHP mL H <sub>2</sub> .L <sup>-1</sup> .d <sup>-1</sup>	Maximum yield H <sub>2</sub> mol H <sub>2</sub> .mol <sup>-1</sup> total carbohydrates	Maximum yield H <sub>2</sub> <sup>a</sup> mmol H <sub>2</sub> .gCOD <sup>-1</sup>	Reference
HBP (35 °C)	Molasses	11.4	68.2	5570	—	5.8	[3]
APBR (25 °C)	Vinasse of sugarcane	24	36.2	509.5	3.2	—	[11]
UASB (55 °C)	Beet Molasses	16	60	5600	—	—	[19]
APBR (55 °C)	Vinasse of sugarcane	12	72.4	1023	2.4	0.7	[18]
USBR2 (55 °C)	Sugarcane Molasse	2	120	2041	0.3	0.45	This study
USBR1 (55 °C)	Sugarcane Molasse	2	120	1123	0.17	0.25	This study

<sup>a</sup> mmolH<sub>2</sub>.gCOD<sup>-1</sup>total applied.

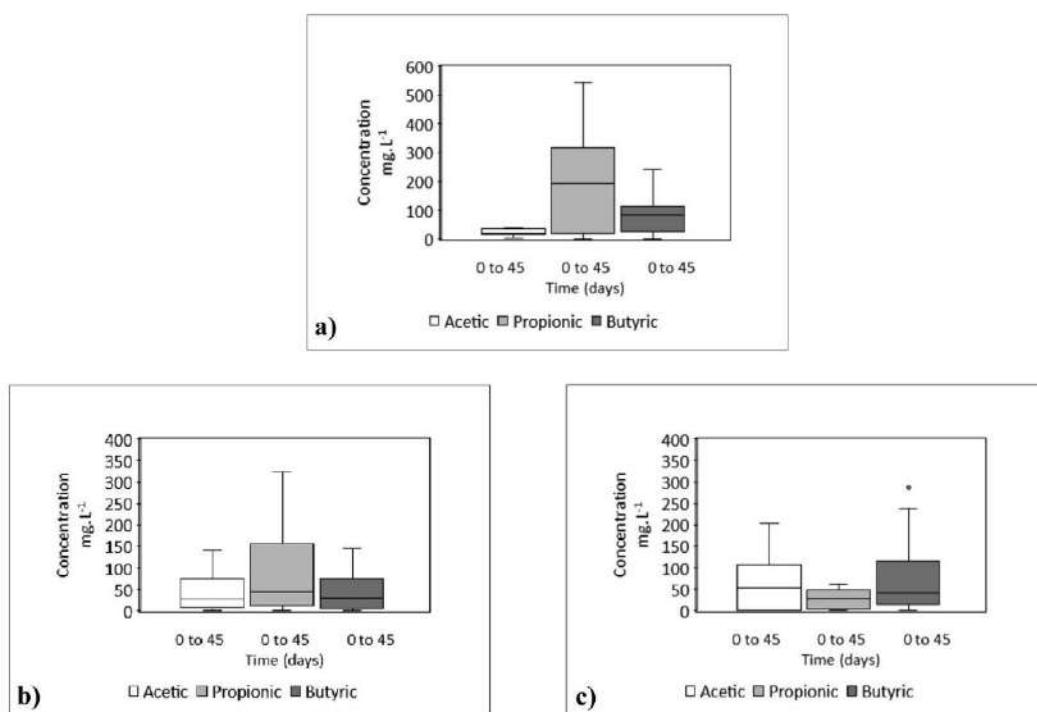


Fig. 4 – Boxplot statistical analyses of organic acids detected in the bioreactors influent (a), USBR1 (b) and USBR2 (c).

work were lower than obtained by other authors (Table 3), showing that the other reactors operated with lower OLR resulted in better conversion of carbohydrates and COD in biogas. Although the other anaerobic systems resulted in a higher hydrogen production and yield than our system, the lower HRT and higher OLR make our setup an interesting technology, since results lower volume of high rate reactor.

#### Evaluation of intermediates

Fig. 4 shows that the influent of the reactor contained acetic, butyric and mainly propionic acids. Propionic acid was metabolized by the microbial community developed in the reactors, especially in USBR2. Hydrogen production was the

result of fermentation of acetic and butyric acids (Fig. 4a–c; Table 4), see equations (3) and (4) [39]. USBR 1 had lower acetic and butyric acids concentrations than USBR 2 (Fig. 4a–c), and this can explain the higher hydrogen production in USBR 2. In fact, acetic and butyric fermentation have been observed as the main fermentative metabolic pathway related to hydrogen production at mesophilic and thermophilic conditions with different sources of wastewater [12,15,18,38,40].

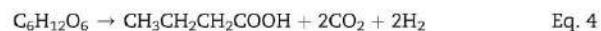
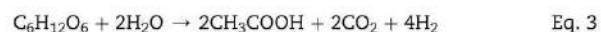


Table 4 – Hydrogen production based on organic acids production of the reactors operated with different organic loading rates (OLR).

OLR (g COD·L <sup>-1</sup> ·d <sup>-1</sup> )	HPR (L H <sub>2</sub> ·day <sup>-1</sup> )	THP (L H <sub>2</sub> ·day <sup>-1</sup> )			$\sum$ THP (L H <sub>2</sub> ·day <sup>-1</sup> )	HPR/ $\sum$ THP (%)
		Acetic	Butyric	Propionic		
<b>USBR1</b>						
30	–	0.9	0.4	–	1.3	–
60	0.9	0.5	0.8	–	1.3	70
120	1.2	1.1	0.5	–	1.6	70
<b>USBR2</b>						
30	–	1.0	1.0	–	1.7	–
60	1.2	1.1	1.9	–	3.0	40
120	2.5	2.4	1.6	–	4.0	60

HPR: hydrogen production rate measured; \*HPR at OLR of 30 was not measured.

THP: theoretical hydrogen production calculated by stoichiometric equations (Eq I, II e III) from the production of HAc and HBu acids, discounting the acids present in the influent. HPr, present in the influent, was consumed in the process.

Our results show that the measured hydrogen production (HPR) was lower than the theoretical hydrogen production (THP) (Table 4). Similar results have been shown before [41], being in the range of 1.2–2.3 mol H<sub>2</sub>/mol glucose [42,43] instead of the theoretical value of 4 mol H<sub>2</sub>/mol glucose when only acid acetic is formed [42]. The ratio HPR/ΣTHP (Table 4) shows a potential in hydrogen recovery higher in USBR1 than in USBR2. Biochemical aspects may be associated with this difference between theoretical and measured hydrogen production. Part of the energy is involved in biomass production and a stoichiometric yield is achievable only under near equilibrium condition, which implies a slow production rate and a low H<sub>2</sub> partial pressure [44,45].

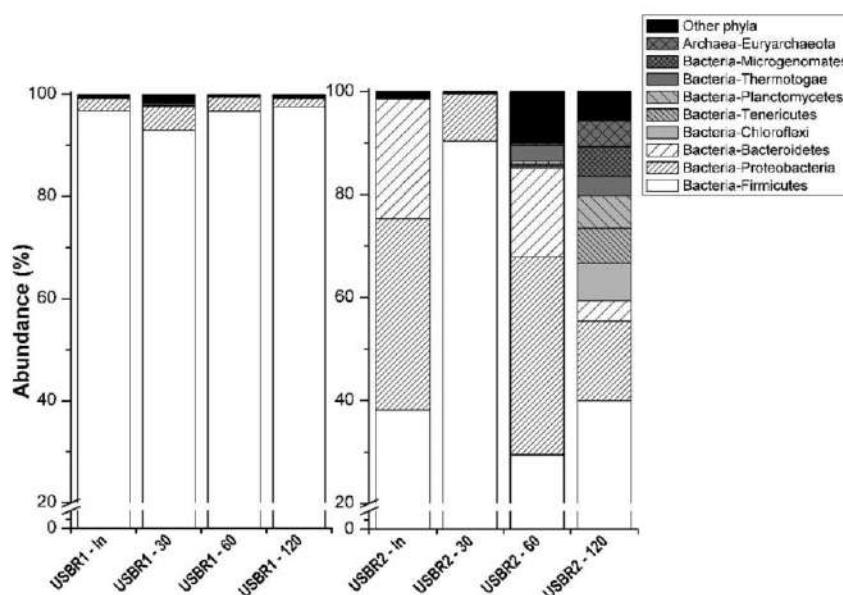
#### Molecular analyses

##### Characterization of microbial community by metagenomic MiSeq technique

A total of 2,746,902 raw reads were obtained from the 16S rRNA sequencing across all samples. From this total, about 2.2% were discarded after dynamic trimming and pair-end joining, resulting in 2,686,698 high quality sequences with an average length of 453bp. These corresponded to 430,576 sequences of the inoculum of USBR1, 470,786 for OLR of 30 g COD.L<sup>-1</sup>.d<sup>-1</sup>, 316,250 for OLR of 60 g COD.L<sup>-1</sup>.d<sup>-1</sup>; and 405,018 for OLR of 120 g COD.L<sup>-1</sup>.d<sup>-1</sup>. For the USBR2, a total of 366,023 sequences from the inoculum were analyzed, plus 269,818 for OLR 30 g COD.L<sup>-1</sup>.d<sup>-1</sup>, 159,793 for 60 g COD.L<sup>-1</sup>.d<sup>-1</sup>, and 268,434 for OLR of 120 g COD.L<sup>-1</sup>.d<sup>-1</sup>.

At the Phylum level, Firmicutes was the most representative group in the auto-fermented vinasse inoculum of USBR1 (96.8%). Firmicutes also predominated and were selected in the USBR1 throughout the operational period for all OLRs applied (Fig. 5) with 93–98% of relative abundance, followed by

Proteobacteria being counter selected (1.5–5%). The sludge of UASB reactor treating vinasse, used as inoculum for the USBR2, showed a more diverse composition. In this inoculum, Firmicutes (38%) and Proteobacteria (37%) were the most abundant Phyla as well, followed by Bacteroidetes (23%) (Fig. 5). In USBR2 starting from OLR of 60 g COD.L<sup>-1</sup>.d<sup>-1</sup> sequences identified to other bacterial Phyla were detected (Chloroflexi; Thermotogae, Planctomycetes; Microgenomates and Tenericutes) that increased in relative abundance at OLR of 120 g COD.L<sup>-1</sup>.d<sup>-1</sup> but never surpassing 7.5% of the total community (Fig. 5). Microorganisms from Phyla Firmicutes, Proteobacteria and Bacteroidetes are dominant in anaerobic bioreactors and those operated for hydrogen production [36,37]. Firmicutes is of functional relevance since it contains members that are involved in many metabolic processes including the degradation of carbohydrates, fatty acids utilization and syntrophic acetate oxidation. Proteobacteria is metabolically diverse with members involved in fermentation and anaerobic respiration. Bacteroidetes is frequently reported in anaerobic reactors and play a role in the hydrolysis of polysaccharides and fermentation of mono- and disaccharides. It is interesting to note the detection of other Phyla in minor frequency, such as Chloroflexi; Thermotogae, Planctomycetes, Microgenomates and Tenericutes. These Phyla that are not reported as dominant in anaerobic reactors, have been recently detected in hydrogen production, with few isolates so far [47–51]. Recent metabolic information inferred from genomes of Microgenomates show a fermentative lifestyle and detection of a membrane bound Ni–Fe hydrogenase suggests the potential to produce hydrogen during fermentation [52,53]. Sequences related with Tenericutes have been recently detected in several anaerobic environments, and metagenomic analysis reveal fermentation metabolism and presence of hydrogenase, also suggesting the potential for hydrogen production [54].



**Fig. 5 – Taxonomic affiliation at level of Phyla of the sequences derived from the inocula (USBR-In) and from the USBRs at OLRs of 30, 60 and 120 g COD.L<sup>-1</sup>.d<sup>-1</sup> (USBR 30, USBR 60, USBR 120). Note the break between 5 and 20%, to improve visualization of the less frequent groups.**

At the class and genera level, Bacilli predominated with high abundance of *Lactobacillus* (~92%) in the auto-fermented vinasse inoculum for USBR1 (Fig. 6). Although the inoculum contained mainly the lactic acid producer *Lactobacillus*, the operational conditions of the USBR1 selected hydrogen producers of Class Clostridia, that were present in the inoculum but in minor frequency (0.1%). The sequences related to hydrogen producers *Thermoanaerobacterium* (average of 70%) and *Clostridium sensu stricto* (summed average of five genera of 18.7%) were the most representative in all OLRs applied to USBR1 and are in accordance with other authors operating reactors for hydrogen production [11,18,36,55]. Sequences related to other microorganisms, such as *Lactobacillus*, *Leuconostoc* and *Sporolactobacillus* were detected in minor frequency. Interestingly, a decrease in *Clostridium sensu stricto* 12 and an increase in *Thermoanaerobacterium* was observed at OLR of 120 g COD.L<sup>-1</sup>.d<sup>-1</sup>, where a decrease in hydrogen production was seen. This suggests that this particular clostridium is involved in hydrogen production.

The inoculum in USBR2 coming from the UASB reactor treating vinasse had a higher phylogenetic diversity than the inoculum of USBR1. The most abundant sequences were of the aerobic Flavobacteria *Empedobacter* (21.7%), sequences related to known hydrogen producers of the Class Clostridia, such as *Thermoanaerobacterium* (19.8%) and *Clostridium sensu stricto* (sum of two major genera 2.1%), and also the gammaproteobacteria *Enterobacter* (17.8%), a hydrogen producer [56]. Other abundant sequences included the metabolic versatile gammaproteobacterial *Trabulsiella* (12.7%) and the bacilli class related to the aerobic spore-forming *Tumebacillus* (4.5%) and *Alicyclobacillus* (4.1%). The operational conditions favored various fermentative microbes during its operation. *Lactobacillus* peaked at OLR of 30 g COD.L<sup>-1</sup>.d<sup>-1</sup> (44.4%) and decreased thereafter, while the proteobacteria *Pseudomonas* peaked at OLR of 60 g COD.L<sup>-1</sup>.d<sup>-1</sup> (15.8%), and could have contributed to

hydrogen production [56]. The hydrogen producers *Clostridium sensu stricto* 1 and 12 also peaked at OLR of 30 g COD.L<sup>-1</sup>.d<sup>-1</sup> (7.5% and 9.3% respectively), while *Thermoanaerobacterium* was counter selected by this community and/or operational conditions (Fig. 6).

At OLR of 120 g COD.L<sup>-1</sup>.d<sup>-1</sup>, we observed the selection of sequences related to Class Clostridia, such as *Gelria* and TTA-B61, and the appearance of sequences related to hydrogen producer *Thermogutta* (Class Planctomycetacia) [57]. The increase of sequences with low frequency of abundance (<5%) with the increase of OLR were classified as other genera (Fig. 6). These sequences are related to members involved direct and indirectly in hydrogen production (Fig. 7).

The taxonomic affiliation revealed a clear difference between USBR1 and USBR2 inocula, and consequently the selection of different microorganisms in each reactor at each OLR applied. In order to investigate the metabolic function from taxonomic data, we assigned the sequences to functional groups based on a manually curated database that maps taxonomic identities to metabolic traits [33]. These authors have shown that functional grouping correlate better with environmental parameters than taxonomic grouping. Fermentation was the predominant metabolic function in both the inoculum of USBR1 and during operation of the reactor at different OLR's, with 94.1%, 68%, 67.6%, and 91.3%, for the inoculum, and OLR of 30, 60 and 120 g COD.L<sup>-1</sup>.d<sup>-1</sup>, respectively (Fig. 8). Despite the apparent initial decrease in fermentation, the taxonomic analysis revealed the selection of different fermentation types. Lactic acid fermentation was prevalent at the inoculum (Fig. 8), with predominance of sequences related to *Lactobacillus* (Fig. 6). Increasing the OLRs resulted in hydrogen production in the reactor due to the enrichment of sequences related to *Clostridium* and *Thermoanaerobacterium* (Fig. 6). Unknown metabolic function was enriched mainly in 30 and 60 g COD.L<sup>-1</sup>.d<sup>-1</sup> (Fig. 8), with high

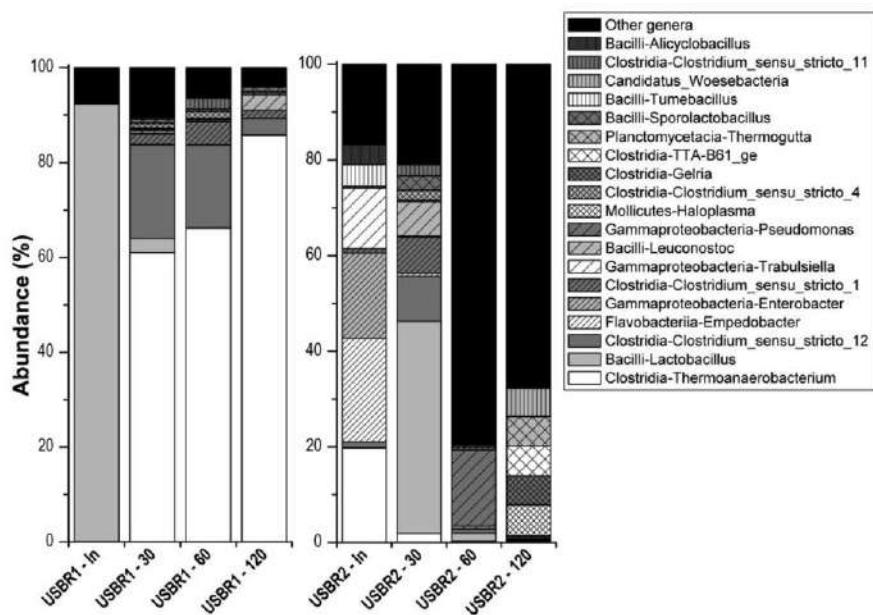


Fig. 6 – Taxonomic affiliation at level of genus of the sequences derived from the inocula (USBR-In) and from the USBRs at OLRs of 30, 60 and 120 g COD.L<sup>-1</sup>.d<sup>-1</sup> (USBR 30, USBR 60, USBR 120).

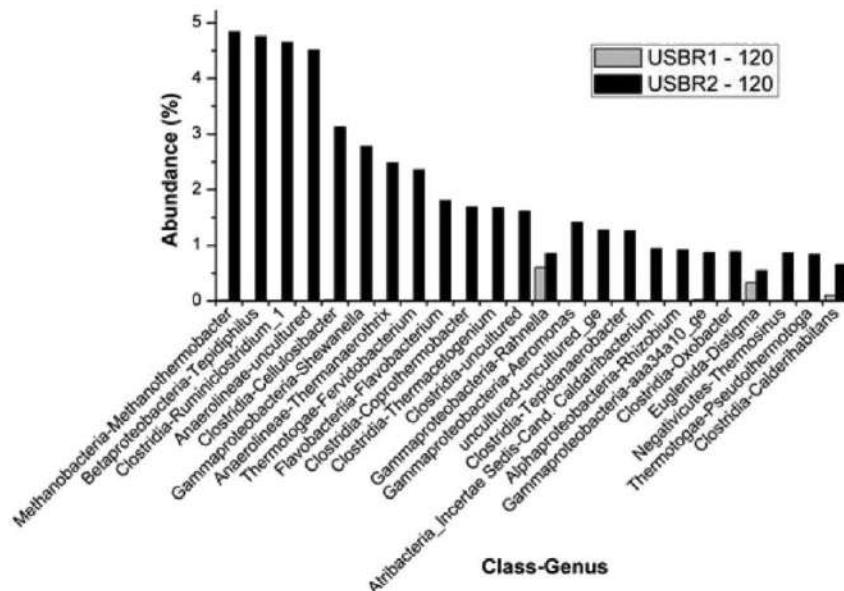


Fig. 7 – Taxonomic affiliation at level of genus of the sequences with relative abundance lower than 5% within the microbial community of USBRs at OLR of 120 g COD.L<sup>-1</sup>.d<sup>-1</sup>.

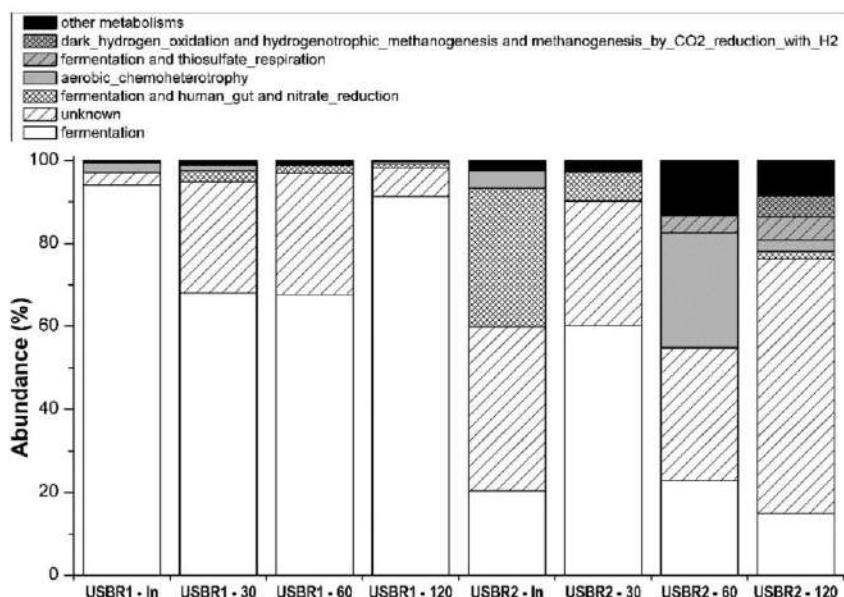


Fig. 8 – Metabolic inferences according to Louca et al. [33] database Faprotax from the inocula (USBR-In) and from the USBRs at OLRs of 30, 60 and 120 g COD.L<sup>-1</sup>.d<sup>-1</sup> (USBR 30, USBR 60, USBR 120). The graph shows stacked values for most abundant functions, representing groups with average abundance across all samples over 0.5%.

hydrogen production (Fig. 3), revealing the importance of physiological characterization of microbial groups. For USBR2, Fig. 8 shows the presence of different metabolic functions in the inoculum, with fermentation (20.4%), fermentation, human gut and reduction of nitrate (33.4%), unknown metabolic function (39.5%) and in minor frequency aerobic respiration (4.3%). Fermentation peaked at an OLR of 30 g COD.L<sup>-1</sup>.d<sup>-1</sup> (60.2%) (Fig. 8) with a predominance of acid lactic

fermentation, since *Lactobacillus* was detected in this OLR (Fig. 6). At OLRs of 60 and 120 g COD.L<sup>-1</sup>.d<sup>-1</sup>, with higher hydrogen production (Fig. 3), the unknown metabolic function was selected (31.9% and 61.3%, respectively, versus 30% at OLR of 30 g COD.L<sup>-1</sup>.d<sup>-1</sup>) (Fig. 8). A lack of information about metabolic functions of microbiota reveals the importance of knowing the physiological characterization of microorganisms in thermophilic anaerobic reactors operated with sugar

cane molasses, since this incomplete knowledge hinders further understanding of these systems. Strategies to overcome this gap are the isolation of microorganisms for physiological studies [58], or the use of other molecular approaches such as metagenome-assembled genomes [59] or single cell genomics [60].

## Conclusions

A structured bed reactor fed with sugar cane molasses and operated with a hydraulic retention time of 2 h with increasing OLR from 30 to 120 g COD.L<sup>-1</sup>.d<sup>-1</sup> was suitable for thermophilic hydrogen production during a period of 45 days. The hydrogen content increased with increasing OLR, up to OLR of 120 g COD.L<sup>-1</sup>.d<sup>-1</sup>.

Organic loading rate, source of inocula and support material influenced the hydrogen production. USBR2 inoculated with sludge from an UASB reactor treating sugar cane vinasse and polyurethane foam as support material achieved a maximum hydrogen production of 2041 mL H<sub>2</sub>d<sup>-1</sup>L<sup>-1</sup> while in USBR1 inoculated with auto-fermented sugarcane vinasse as inoculum and polyethylene low density as support material, a maximum hydrogen production of 1123 mL H<sub>2</sub>d<sup>-1</sup>L<sup>-1</sup> was achieved.

Inoculum of USBR2 was taxonomically and functionally more diverse than the inoculum of USBR1 with sequences related to *Empedobacter*, *Thermoanaerobacterium*, *Clostridium sensu stricto*, *Enterobacter* and *Trabulsiella* while in inoculum of USBR1 fermentation prevailed with sequences related to *Lactobacillus*. Operational conditions selected *Thermoanaerobacterium* and *Clostridium sensu stricto* in USBR1, while in USBR2, besides these microorganisms, *Lactobacillus*, *Pseudomonas* and *Thermotoga* were also predominant. Microorganisms with lower abundance (<5%) appeared to be involved directly and indirectly in hydrogen production. In addition to fermentation, unknown metabolism was relevant mainly in USBR2, revealing the importance of physiological characterization of microbial groups.

## Acknowledgements

This research was funded by grants from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (Proc. 2012/51496-3) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) of Brazil.

## Appendix A. Supplementary data

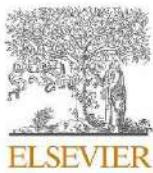
Supplementary data related to this article can be found at <https://doi.org/10.1016/j.ijhydene.2019.08.180>.

## REFERENCES

- [1] União das Industrias de cana de açúcar. (accessed 05 of may of 2019).
- [2] Otero-Rambla MA, García R, Pérez MC, JÁ Martínez, Vasallo MC, Saura G, Bello D. Producción de bioetanol a partir de mezclas de jugos-melazas de caña de azúcar ICIDCA. núm. 1, enero-abril. Sobre los Derivados de la Caña de Azúcar 2009;XLIII:17–22. Instituto Cubano de Investigaciones de los Derivados de la Caña de Azúcar. Disponible en:<http://www.redalyc.org/articulo.oa?id=223120657003>%20ISSN%200138-6204%20electrónico:%201025-3076>
- [3] Ren N, Li J, Li B, Wang Y, Liu S. Biohydrogen production from molasses by anaerobic fermentation with a pilot-scale bioreactor system. Int J Hydrogen Energy 2006;31:2147–57. <https://doi.org/10.1016/j.ijhydene.2006.02.011>.
- [4] Li J, Li B, Zhu G, Ren N, Bo L, He J. Hydrogen production from diluted molasses by anaerobic hydrogen producing bacteria in an anaerobic baffled reactor (ABR). Int J Hydrogen Energy 2007;32:3274–83. <https://doi.org/10.1016/j.ijhydene.2007.04.023>.
- [5] Wang B, Yongfeng L, Nan-Qi R. Biohydrogen from molasses with ethanol-type fermentation: effect of hydraulic retention time. Int J Hydrogen Energy 2013;38:4361–7. <https://doi.org/10.1016/j.ijhydene.2013.01.120>.
- [6] Gavala H, Skiadas I, Ahring B. Biological hydrogen production in suspended and attached growth anaerobic reactor systems. Int J Hydrogen Energy 2006;31:1164–75. <https://doi.org/10.1016/j.ijhydene.2005.09.009>.
- [7] Fuesse LT, Kiyuna LSM, Ferraz Jr ADN, Persinoti GF, Squina FM, Garcia ML, Zaiat M. Thermophilic two-phase anaerobic digestion using an innovative fixed-bed reactor for enhanced organic matter removal and bioenergy recovery from sugarcane vinasse 2017; Appl Energy 189:480–491. <https://doi.org/10.1016/j.apenergy.2016.12.071>.
- [8] Camiloti PR, Mockaitis G, Rodrigues JAD, Damianovic MHRZ, Foresti E, Zaiat M. Innovative anaerobic bioreactor with fixed-structured bed (ABFSB) for simultaneous sulfate reduction and organic matter removal. J Chem Technol Biotechnol 2014;89:1044–50. <https://doi.org/10.1002/jctb.4199>.
- [9] Keskin T, Giusti L, Azbar N. Continuous biohydrogen production in immobilized biofilm system versus suspended cell culture. Int J Hydrogen Energy 2012;37:1418–24. <https://doi.org/10.1016/j.ijhydene.2011.10.013>.
- [10] Fernandes BS, Saavedra NK, Maintinguier SI, Sette LD, Oliveira VM, Varesche MBA, et al. The effect of biomass immobilization support material and bed porosity on hydrogen production in an upflow anaerobic packed-bed bioreactor. Appl Biochem Biotechnol 2013;170:1348–66. <https://doi.org/10.1007/s12010-013-0262-7>.
- [11] Ferraz Jr ADN, Etchebehere C, Zaiat M. Mesophilic hydrogen production in acidogenic packed-bed reactors (APBR) using raw sugarcane vinasse as substrate: influence of support materials. Anaerobe 2015;34:94–105. <https://doi.org/10.1016/j.janaerobe.2015.04.008>.
- [12] Muria P, Marinsek-Logar L, Petar D, Pintara A. Influence of support materials on continuous hydrogen production in anaerobic packed-bed reactor with immobilized hydrogen producing bacteria at acidic conditions. Enzym Microb Technol 2018;111:87–96. <https://doi.org/10.1016/j.enzmictec.2017.10.008>.
- [13] Anzola Rojas MP, Fonseca SG, Silva CC, Oliveira VM, Zaiat M. The use of the carbon/nitrogen ration and specific organic loading rate as tools for improving biohydrogen production in fixed-bed reactors. Biotechnol Rep 2015;5:46–54. <https://doi.org/10.1016/j.btre.2014.10.010>.
- [14] Anzola-Rojas MP, Zaiat M, Wever H. Improvement of hydrogen production via ethanol-type fermentation in an anaerobic down-flow structured bed reactor. Bioresour Technol 2016;202:42–9. <https://doi.org/10.1016/j.biortech.2015.11.084>.

- [15] Penteado ED, Lazaro CZ, Sakamoto IK, Zaiat M. Influence of seed sludge and pretreatment method on hydrogen production in packed-bed anaerobic reactors. *Int J Hydrogen Energy* 2013;38:6137–45. <https://doi.org/10.1016/j.ijhydene.2012.01.067>.
- [16] Pecorini I, Baldi F, Iannelli R. Biochemical hydrogen potential tests using different inocula. *Sustainability* 2019;11(622):1–17. Advances in Biorefining of Biowaste, <https://doi.org/10.3390/su11030622>.
- [17] Sigurbjörnsdóttir MA, Orlygsson J. Combined hydrogen and ethanol production from sugars and lignocellulosic biomass by *Thermoanaerobacterium AK54*, isolated from hot spring. *Appl Energy* 2012;97:785–91. <https://doi.org/10.1016/j.apenergy.2011.11.035>.
- [18] Ferraz Jr ADN, Wensel J, Etchebehere C, Zaiat M. Effect of organic loading rate on hydrogen production from sugarcane vinasse in thermophilic acidogenic packed bed reactors. *Int J Hydrogen Energy* 2014;39:16852–62. <https://doi.org/10.1016/j.ijhydene.2014.08.017>.
- [19] Kongjan P, O-Thong S, Angelidaki I. Hydrogen and methane production from desugared molasses using a two-stage thermophilic anaerobic process. *Eng Life Sci* 2013;13:118–25. <https://doi.org/10.1002/elsc.201100191>.
- [20] Roy S, Vishnuvardhan M, Das D. Continuous thermophilic biohydrogen production in packed bed reactor. *Appl Energy* 2014;31(136):51–8. <https://doi.org/10.1016/j.apenergy.2014.08.031>.
- [21] Godoi L, Damianovic MHRZ, Foresti E. Sulfidogenesis interference on methane production from carbohydrate-rich wastewater. *Water Sci Technol* 2015;74:1644–52. <https://doi.org/10.2166/wst.2015.383>.
- [22] APHA, AWWA, WEF. Standard methods for the examination of water and wastewater. 21st ed. Washington D.C.: APHA; 2005.
- [23] Dubois SM, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric methods for determination of sugar and related substance. *Anal Chem* 1956;228:13–21. <https://doi.org/10.1021/ac60111a017>.
- [24] Ripley LE, Boyle WC, Converse JC. Improved alkalimetric monitoring for anaerobic digestion of high strength wastes. *J Water Pollut Control Fed* 1986;58:406–11. <https://www.jstor.org/stable/25042933>.
- [25] Saia FT, Damianovic MHZ, Cattony EB, Brucha G, Foresti E, Vazoller RF. Anaerobic biodegradation of pentachlorophenol in a fixed-film reactor inoculated with polluted sediment from Santos São Vicente Estuary, Brazil. *Appl Microbiol Biotechnol* 2007;75:665–72. <https://link.springer.com/article/10.1007/s00253-007-0841-z>.
- [26] Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glöckner FO. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res* 2013;41:1–11. <https://doi.org/10.1093/nar/gks808>.
- [27] Cox MP, Peterson DA, Biggs PJ. SolexaQA: at-a-glance quality assessment of Illumina second-generation sequencing data. *BMC Bioinf* 2010;11:485. <https://doi.org/10.1186/1471-2105-11-485>.
- [28] Masella AP, Bartram AK, Truszkowski JM, Brown DG, Neufeld JD. PANDAseq: paired-end assembler for illumina sequences. *BMC Bioinf* 2012;13:31. <https://doi.org/10.1186/1471-2105-13-31>.
- [29] Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 2011;27:2194–200. <https://doi.org/10.1093/bioinformatics/btr381>.
- [30] Mahé F, Rogness T, Quince C, Vargas C de, Dunthorn M. Swarm: robust and fast clustering method for amplicon-based studies. *PeerJ* 2014;2: e593, <https://doi.org/10.7717/peerj.593>.
- [31] Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thalinger GG, Horn DJV, Weber CF. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* 2009;75:7537–41. <https://doi.org/10.1128/AEM.01541-09>.
- [32] Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 2013;41:D590–6. 2013, <https://doi.org/10.1093/nar/gks1219>.
- [33] Louca S, Parfrey LW, Doebele M. Decoupling function and taxonomy in the global ocean microbiome. *Science* 2016;353:1272–7. <https://doi.org/10.1126/science.aaf4507>.
- [34] Parks DH, Tyson GW, Hugenholtz P, Beiko RG. STAMP: statistical analysis of taxonomic and functional profiles. *Bioinformatics* 2014;30:3123–4. <https://doi.org/10.1093/bioinformatics/btu494>.
- [35] Fang HHP, Zhang T, LI C. Characterization of Fe-hydrogenase genes diversity and hydrogen-producing population in an acidophilic sludge. *J Biotechnol* 2006;126:357–64. <https://doi.org/10.1016/j.biote.2006.04.023>.
- [36] Ottaviano LM, Ramos LR, Botta LS, Varesche MBA, Silva EL. Continuous thermophilic hydrogen production from cheese whey powder solution in an anaerobic fluidized bed reactor: effect of hydraulic retention time and initial substrate concentration. *Int J Hydrogen Energy* 2017;42:4848–60. <https://doi.org/10.1016/j.ijhydene.2016.11.168>.
- [37] Carosia MF, Reis CM, Sakamoto IK, Varesche MBA, Silva EL. Influence of C/P and C/N ratios and microbial characterization in hydrogen and ethanol production in an anaerobic fluidized bed reactor. *Int J Hydrogen Energy* 2017;42:9600–10. <https://doi.org/10.1016/j.ijhydene.2017.01.127>.
- [38] Han W, Wang B, Zhou Y, Wang D, Wang Y, Yue L, et al. Fermentative hydrogen production from molasses wastewater in a continuous mixed immobilized sludge reactor. *Bioresour Technol* 2012;110:219–23. <https://doi.org/10.1016/j.biortech.2012.01.057>.
- [39] Antonopoulou G, Gavala HN, Skiadas IV, Angelopoulos K, Lyberatos G. Biofuels generation from sweet sorghum: fermentative hydrogen production and anaerobic digestion of the remaining biomass. *Bioresour Technol* 2008;99:110–9. <https://doi.org/10.1016/j.biortech.2006.11.048>.
- [40] Braga AFM, Ferraz Jr ADN, Zaiat M. Thermophilic biohydrogen production using a UASB reactor: performance during long-term operation. *J Chem Technol Biotechnol* 2016;91:967–76. <https://doi.org/10.1002/jctb.4665>.
- [41] Kalia VC, Purohit HJ. Microbial diversity and genomics in aid of bioenergy. *J Ind Microbiol Biotechnol* 2008;35:403–19. <https://doi.org/10.1007/s10295-007-0300-y>.
- [42] Angenent LT, Karim K, Al-Dahhan M, Wrenn BA, Domínguez-Espínosa R. Production of bioenergy and biochemicals from industrial and agricultural wastewater. *Trends Biotechnol* 2004;22:477–85. <https://doi.org/10.1016/j.tibtech.2004.07.001>.
- [43] Logan BE. Extracting hydrogen and electricity from renewable resources. *Environ Sci Technol* 2004;38:160–7. <https://doi.org/10.1021/es040468s>.
- [44] Woodward JI, Orr M, Cordray K, Greenbaum E. Enzymatic Production of Biohydrogen. *Nature* 2000;405:1014–5. <https://doi.org/10.1038/35016633>.

- [45] Hallenbeck PC, Benemann J. Biological hydrogen production; fundamentals and limiting processes. *Int J Hydrogen Energy* 2002;27:1185–93. [https://doi.org/10.1016/S0360-3199\(02\)00131-3](https://doi.org/10.1016/S0360-3199(02)00131-3).
- [47] Rivière D, Desvignes V, Pelletier E, Chaussonnerie S, Guermazi S, Weissenbach J, Li T, Camacho P, Sghir A. Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge. *ISME J* 2009;3:700–14. <https://doi.org/10.1038/ismej.2009.2>.
- [48] Grégoire P, Fardeau ML, Joseph M, Guasco S, Hamaide F, Biasutti S, Michotey V, Bonin P, Ollivier B. Isolation and characterization of *Thermaanaerothrix daxensis* gen. nov., sp. nov., a thermophilic anaerobic bacterium pertaining to the phylum "Chloroflexi", isolated from a deep hot aquifer in the Aquitaine Basin. *Syst Appl Microbiol* 2011;34:494–7. <https://doi.org/10.1016/j.syapm.2011.02.004>.
- [49] Maune MW, Tanner RS. Description of *Anaerobaculum hydrogeniformans* sp. nov., an anaerobe that produces hydrogen from glucose, and emended description of the genus *Anaerobaculum*. *Int J Syst Evol Microbiol* 2012;62:832–8. <https://doi.org/10.1099/ijss.0.024349-0>.
- [50] Liu D, Liu Y, Men X, Guo Q, Guo R, Qiu Y. Isolation and characterization of *Thermopirellula anaerolimosa* gen. nov., sp. nov., an obligate anaerobic hydrogen-producing bacterium of the phylum Planctomycetes. *Acta Microbiol Sin* 2012;52:994–1001. PMID: 23173436.
- [51] Sivarajan A, Shanmugam Sundaram T, Thirumalairaj J, Balagurunathan R. Production and optimization of biohydrogen from saccharolytic actinobacterium, *Streptomyces rubiginosus* (SM16), using sugarcane molasses. *Biofuels* 2017;8:717–23. <https://doi.org/10.1080/17597269.2016.1257317>.
- [52] Wrighton KC, Thomas BC, Sharon I, Miller CS, Castelle CJ, VerBerkmoes NC, Wilkins MJ, Hettich RL, Lipton MS, Williams KH, Long PE. Fermentation, hydrogen, and sulfur metabolism in multiple uncultivated bacterial phyla. *Science* 2012;337:1661–5. <https://doi.org/10.1126/science.1224041>.
- [53] Solden L, Lloyd K, Wrighton K. The bright side of microbial dark matter: lessons learned from the uncultivated majority. *Curr Opin Microbiol* 2016;31:217–26. <https://doi.org/10.1016/j.mib.2016.04.020>.
- [54] Skennerton CT, Haroon MF, Briegel A, Shi J, Jensen GJ, Tyson GW, Orphan VJ. Phylogenomic analysis of *Candidatus 'Izimaplasma'* species: free-living representatives from a Tenericutes clade found in methane seeps. *ISME J* 2016;10:2679–92. <https://doi.org/10.1038/ismej.2016.55>.
- [55] Ratti RP, Delforno TP, Sakamoto IK, Varesche MB. Thermophilic hydrogen production from sugarcane bagasse pretreated by steam explosion and alkaline delignification. *Int J Hydrogen Energy* 2015;40:6296–306. <https://doi.org/10.1016/j.ijhydene.2015.03.067>.
- [56] Kumar N, Das D. Enhancement of hydrogen production by *Enterobacter cloacae* IIT-BT 08. *Process Biochem* 2000;35:589–93. [https://doi.org/10.1016/S0032-9592\(99\)00109-0](https://doi.org/10.1016/S0032-9592(99)00109-0).
- [57] Slobodkina GB, Kovaleva OL, Miroshnichenko ML, Slobodkin AI, Kolganova TV, Novikov AA, van Heerden E, Bonch-Osmolovskaya EA. *Thermogutta terrifontis* gen. nov., sp. nov. and *Thermogutta hypogea* sp. nov., thermophilic anaerobic representatives of the phylum Planctomycetes. *Int J Syst Evol Microbiol* 2015;65:760–5. <https://doi.org/10.1099/ijss.0.000009>.
- [58] Prakash Om, Shouch Y, Jangid K, Kostka JE. Microbial cultivation and the role of microbial resource centers in the omics era. *Appl Microbiol Biotechnol* 2013;97:51–62. <https://doi.org/10.1007/s00253-012-4533-y>.
- [59] Parks DH, Rinke C, Chuvochina M, Chaumeil PA, Woodcroft BJ, Evans PN, Hugenholtz P, Tyson GW. Recovery of nearly 8,000 metagenome-assembled genomes substantially expands the tree of life. *Nat Microbiol* 2017;2:1533–42. <https://doi.org/10.1038/s41564-017-0012-7>.
- [60] Rinke C, Schwientek P, Sczyrba A, Ivanova NN, Anderson IJ, Cheng JF, Darling A, Malfatti S, Swan BK, Gies EA, Dodsworth JA. Insights into the phylogeny and coding potential of microbial dark matter. *Nature* 2013;499:431–7. <https://doi.org/10.1038/nature12352>.



Contents lists available at ScienceDirect

## Renewable and Sustainable Energy Reviews

journal homepage: <http://www.elsevier.com/locate/rser>

## Biofuel production from sugarcane molasses in thermophilic anaerobic structured-bed reactors

R.S. Vilela<sup>a,1</sup>, L.T. Fuess<sup>a,b,\*1</sup>, F.T. Saia<sup>c,1</sup>, C.R.M. Silveira<sup>c</sup>, C.A. Oliveira<sup>a</sup>, P.A. Andrade<sup>d</sup>, A. Langenhoff<sup>e</sup>, B. van der Zaan<sup>f</sup>, F. Cop<sup>c</sup>, G.B. Gregoracci<sup>c</sup>, M.H.R.Z. Damianovic<sup>a</sup>

<sup>a</sup> Biological Process Laboratory, São Carlos School of Engineering, University of São Paulo, Av. João Dagnone, 1100, Santa Angelina, 13563-120, São Carlos, SP, Brazil

<sup>b</sup> Chemical Engineering Department, Polytechnic School, University of São Paulo, Av. Prof. Lineu Prestes, 580, Bloco 18 – Conjunto das Químicas, 05508-000, São Paulo, SP, Brazil

<sup>c</sup> Institute of Marine Sciences, Federal University of São Paulo, Av. Dr. Carvalho de Mendonça, 144, Encruzilhada, 11070-102, Santos, SP, Brazil

<sup>d</sup> Department of Soil Science, "Luiz de Queiroz" College of Agriculture, University of São Paulo, Av. Pádua Dias, 11, 13418-900, Piracicaba, SP, Brazil

<sup>e</sup> Department of Environmental Technology, Wageningen University & Research, PO Box 17, 6700 EV, Wageningen, the Netherlands

<sup>f</sup> Department of Subsurface and Groundwater Systems Deltares, PO Box 85467, 3508 AL, Utrecht, the Netherlands

## ARTICLE INFO

## ABSTRACT

**Keywords:**  
 Sugarcane biorefinery  
 Two-stage biodigestion  
 Long-term biohydrogen production  
 Metagenomic and qPCR analyses  
 MiSeq Illumina  
 Energetic potential assessment

This work presents an alternative bioenergy-related management approach for sugarcane molasses through the application of anaerobic digestion (AD) in a two-stage continuous thermophilic (55 °C) system to produce biohydrogen (bioH<sub>2</sub>) and methane. The performance of the acidogenic stage (RH<sub>2</sub>) was assessed by maintaining a continuous and high organic loading rate (OLR; 120 kg COD m<sup>-3</sup> d<sup>-1</sup>), whilst the robustness of the methanogenic stage (RCH<sub>4</sub>) was investigated based on the increase of the OLR (1.0–25.2 kg COD m<sup>-3</sup> d<sup>-1</sup>). Molecular analyses and an energetic assessment were also conducted, to provide a holistic understanding of the two-stage AD system. Long-term bioH<sub>2</sub> production was achieved at low pH values (~4.0) in RH<sub>2</sub> by the co-fermentation of lactate and acetate, and a positive correlation between *Clostridium* and *Leuconostoc* genera was identified. Efficient methane production (323–350 NmL CH<sub>4</sub> g<sup>-1</sup> COD) was only observed at low OLR (1.0–2.3 kg COD m<sup>-3</sup> d<sup>-1</sup>) in RCH<sub>4</sub>, although high COD removal levels (>70%) were observed at all operational conditions. Metabolite and molecular analyses indicated inefficient syntrophic and acetoclastic activities (accumulation of acetate, propionate and lactate), indicating that hydrogenotrophic methanogenesis was the prevailing methane-producing pathway in RCH<sub>4</sub>, specifically by the *Methanothrixobacter* genus. Finally, the energetic potential (8560 kJ kg<sup>-1</sup> COD<sub>applied</sub>) of molasses outperformed the ones of vinasse by at least 25%, indicating that the high availability of biodegradable organic matter in molasses requires a low OLR to offer efficient bioenergy recovery levels.

## 1. Introduction

There is much discussion about energy availability, as well as investments to replace fossil fuels with alternative (and cleaner) energy sources, which include solar, wind, geothermal, tidal and hydro power [1]. Simultaneously to this, there is an ongoing search for minimizing industrial waste generation, with alternative processing approaches.

Such approaches focus on the mitigation of environmental problems, while generating profits for companies, solving local energy demands, generating more jobs, and meeting environmental legislation [2]. Ethanol and sugar production by-products from sugarcane processing, such as bagasse, vinasse and molasses, are examples of industrial waste that can be reused, and the Brazilian case deserves special valorization, as Brazil is the largest producer of sugarcane and its derivatives.

Currently, different approaches are applied in Brazil for the

\* Corresponding author. Biological Process Laboratory, São Carlos School of Engineering, University of São Paulo, Av. João Dagnone, 1100, Santa Angelina, 13563-120, São Carlos, SP, Brazil.

E-mail addresses: [vilela.rogerio@gmail.com](mailto:vilela.rogerio@gmail.com) (R.S. Vilela), [ltfuess@alumni.usp.br](mailto:ltfuess@alumni.usp.br), [ltfuess@alumni.usp.br](mailto:ltfuess@alumni.usp.br) (L.T. Fuess), [ftsai@yahoo.com.br](mailto:ftsai@yahoo.com.br) (F.T. Saia), [cristiane.arruda.oliveira@usp.br](mailto:cristiane.arruda.oliveira@usp.br) (C.A. Oliveira), [pedro890@hotmail.com](mailto:pedro890@hotmail.com) (P.A. Andrade), [allette.langenhoff@wur.nl](mailto:allette.langenhoff@wur.nl) (A. Langenhoff), [bas.vanderzaan@deltas.nl](mailto:bas.vanderzaan@deltas.nl) (B. van der Zaan), [fabiocferreira@gmail.com](mailto:fabiocferreira@gmail.com) (F. Cop), [gustavo.biomed@yahoo.com](mailto:gustavo.biomed@yahoo.com) (G.B. Gregoracci), [mdamianovic@sc.usp.br](mailto:mdamianovic@sc.usp.br) (M.H.R.Z. Damianovic).

<sup>1</sup> Shared first authorship.

List of abbreviations and symbols:	
AD	anaerobic digestion
AnSTBR	anaerobic structured-bed reactor
BFR	biogas flow rate
bioH <sub>2</sub>	biohydrogen
BOD	biochemical oxygen demand
CCA	canonical correspondence analysis
CHt	total carbohydrates
COD	chemical oxygen demand
COD <sub>inf</sub>	COD applied in the reactors
EC <sub>CHt</sub>	CHt conversion efficiency
EP	energetic potential (generic nomenclature)
EP <sub>CH4</sub>	energetic potential of methane
EP <sub>global</sub>	global energetic potential
EP <sub>H2</sub>	energetic potential of bioH <sub>2</sub>
ER <sub>COD</sub>	COD removal efficiency
ER <sub>CH4</sub>	COD removal efficiency (methanogenic phase)
ER <sub>H2</sub>	COD removal efficiency (acidogenic phase)
fCH <sub>4</sub>	methane proportion in biogas
fH <sub>2</sub>	hydrogen proportion in biogas
GC/TCD	gas chromatography with thermal conductivity detector
HAc	acetate
HBu	butyrate
HCi	citric acid
HCO <sub>3</sub> <sup>-</sup>	bicarbonate ion
HFo	formate
HLa	lactate
HMa	malic acid
HPLC	high performance liquid chromatography
HPr	propionate
HRT	hydraulic retention time
HSu	succinic acid
HY	hydrogen yield
IA	intermediate alkalinity
LAB	lactic acid bacteria
LHV <sub>CH4</sub>	lower heating value of methane
LHV <sub>H2</sub>	lower heating value of hydrogen
MB <sub>COD</sub>	COD-based mass balance
McrA	methanogenic Archaea
MY	methane yield
NaHCO <sub>3</sub>	sodium bicarbonate
OLR	organic loading rate
OTU	operational taxonomic unit
PA	partial alkalinity
PU	polyurethane
qPCR	quantitative polymerase chain reaction
RCH <sub>4</sub>	methanogenic reactor
RenovaBio	(Brazilian) National Biofuel Policy
RH <sub>2</sub>	acidogenic reactor
rRNA	ribosomal RNA
SMP <sub>eff</sub> , SMP	SMP soluble phase metabolite
SO <sub>4</sub> <sup>2-</sup>	sulfate
TKN	total Kjeldahl nitrogen
UASB	upflow anaerobic sludge blanket reactor
VFA	volatile fatty acids
VHPR	volumetric hydrogen production rate
VMPR	volumetric methane production rate

management of the various sugarcane by-product. Bagasse is directed to boilers to produce bioelectricity and steam, to supply the industrial plant itself, and to provide additional revenues through bioelectricity sales to the grid [3]. Vinasse is used in the fertirrigation of sugarcane fields, aiming to recycle water and potassium. However, the potential environmental impacts associated with this practice [4] indicate the need to properly manage vinasse. The application of anaerobic digestion (AD) to these waste streams has been addressed as the most attractive approach to both achieve environmental compliance and bioenergy production [5–7]. This is in line with the implementation of the National Biofuel Policy (RenovaBio) in Brazil, which stimulates the application and commercialization of renewable energy from alternative sources [8], such as bagasse and vinasse.

Molasses is another sugar production by-product and is characterized as a carbohydrate-rich substrate which could also play a relevant role in anaerobic digestion systems. Although molasses is conventionally used as substrate in ethanol production, its high biodegradability suggests a high suitability to the application of AD as well. This approach could lead to scenarios in which ethanol production is replaced by biogas as the target product, providing higher flexibility to sugarcane biorefineries as bioenergy suppliers. For example, methane-rich biogas may be used in bioelectricity/steam generation [9,10], and in biomethane production (purified biogas, i.e., methane content higher than 96.5%; [11]), which can be sold to natural gas grids, or used as automotive fuel [6,12].

To achieve this, an efficient energy recovery from molasses in anaerobic systems depends directly on separation of sequential processes, i.e. the separation of acidogenesis and methanogenesis in independent sequential processing units. This approach brings numerous advantages, such as the simultaneous provision of optimal operating conditions to different microbial populations, namely, fermentative bacteria and methanogens, and the possibility to recover biohydrogen (bioH<sub>2</sub>) in the acidogenic stage [13–15]. In particular, during the

operation of such a two-stage AD system most of substrate (primarily sugars in the case of molasses) fermentation occurs in the prior acidogenic unit, which minimizes the establishment of stressing conditions to the methanogenic biomass. This will result in more stable methanogenesis.

Recent studies have applied two-stage AD in the processing of sugarcane-derived by-products, such as vinasse [13,14,16] and molasses [17–19], usually highlighting the application of thermophilic conditions in the reactors. Thermophilic AD systems tend to efficiently withstand higher organic loading rates (OLR) than mesophilic ones (almost twice as high) in stable operating conditions [20], which leads to more compact processing units. Additional advantages are reflected in the acidogenic stage, as bioH<sub>2</sub> production is favored at higher temperatures [21–23], due to metabolic shifts by the fermentative populations and lower gas solubility. In particular, the maintenance of thermophilic AD systems in sugarcane biorefineries may be easily achieved, as the steam produced in boilers through bagasse burning provides an efficient source of heat.

Thermophilic AD of sugarcane molasses gives the possibility to maximize the energy (biogas) production from sugars. Although recent studies showed the bioH<sub>2</sub>-producing potential of molasses [17–19], the hardly any information is available on application of fermented molasses in methanogenic systems. Similarly, the definition of proper reactor configurations comprehends a relevant point. Using the anaerobic structured-bed reactor (AnSTBR) as an alternative to conventional sludge-blanket and packed-bed systems has recently been pointed as a possibility to better control biomass washout in the application of high OLR in vinasse methanogenesis [14,24]. This particular reactor configuration has been also successfully applied in acidogenic systems [16,18], which motivates investigating its potential in high-rate methanogenic systems.

Finally, the role of the microbial populations acting in molasses anaerobic conversion and the energetic potential of the processes needs

to be considered. For example, the thermophilic fermentation of sugarcane-derived by-products seems to be strictly dependent on the *Thermoanaerobacterium* and *Clostridium* genera, as previously observed for vinasse [25] and molasses [18,19,26]. However, little is known of the methanogenic populations that are involved in second-stage reactors. The predominant type of fermentation will directly impact the microbial populations in the methanogenic stage, depending mainly on the relevance of acetic-type fermentation and carbohydrate conversion efficiency. Considering energetic aspects, it is imperative to understand the potential gains associated with the AD of molasses, as it will directly encourage the implementation of full-scale plants. Previous studies on sugarcane vinasse AD indicated important gains on the application of two-stage AD [9,13], e.g. a 25.7% increase in energy production coupled to a 50% decrease in sodium bicarbonate ( $\text{NaHCO}_3$ ) dosing, so that similar assessments must be carried out within the molasses biodigestion context.

This study addresses the recovery of bio $\text{H}_2$  and methane from the two-stage thermophilic (55 °C) AD of sugarcane molasses. Important aspects that are studied included the use of structured-bed reactors and thermophilic temperature conditions, as well as the characterization of both the microbial communities and the energetic potential of AD. First, the process performance was investigated in long-term operating periods (250 days) to identify conversion pathways in acidogenesis and the stability of methanogenesis. Secondly, microbial characterization of both acidogenic and methanogenic stages was carried out, to link process performance with the primary communities established within each AnSTBR. As third, a preliminary energetic assessment was conducted to determine the contribution of molasses AD for bioenergy generation in sugarcane biorefineries. This study is the first case to provide a holistic understanding of molasses AD, addressing both fundamental (definition of suitable operating conditions) and applied (potential energy gains from biogas) aspects in detail. The obtained results are of great value to encourage the application of efficient full-scale anaerobic energy-producing systems, providing figures to diversify the exploitation of sugarcane-derived substrates towards an effective application of the biorefinery concept.

## 2. Material and methods

### 2.1. Fresh molasses characterization

Sugarcane molasses was obtained in a full-scale sugarcane biorefinery located in Pradópolis, SP, Brazil. The collected samples were stored at 4 °C prior to feed preparation. Basic compositional aspects of the molasses included; chemical oxygen demand (COD) = 872 mg g<sup>-1</sup>molasses, total carbohydrates concentration (CHt) = 477 mg g<sup>-1</sup>molasses, biochemical oxygen demand (BOD) = 399 mg g<sup>-1</sup>molasses, total Kjeldahl nitrogen (TKN) = 26.9 mg g<sup>-1</sup>molasses and sulfate ( $\text{SO}_4^{2-}$ ) concentration = 7.0 mg g<sup>-1</sup>molasses. Molasses was diluted to desired COD levels with tap water prior to feeding the acidogenic reactor.

### 2.2. Bioreactors and experimental set-up

Two bench-scale (1.65 L each) AnSTBR of tubular acrylic, filled with polyurethane (PU) foam strips for biomass attachment, were used as the processing units. PU strips were placed vertically (Fig. 1), providing, simultaneously, high surface area and high void indices. The reactors were placed in a thermostatic chamber (Model 410-DRE, Nova Ética, Vargem Grande Paulista, SP, Brazil) set to 55 °C, whilst reactor feeding was controlled by peristaltic pumps (Model Minipuls Evolution, Gilson, Inc., Middleton, WI, USA). Gas meters (model MGC-1 V30; Dr.-Ing. Ritter Apparatenbau GMBH & CO. KG, Bochum, Germany) were directly coupled to the headspace of the systems to continuously monitor biogas production.

### 2.3. Reactor inoculation and operating conditions

#### 2.3.1. Acidogenic stage

The acidogenic AnSTBR (RH<sub>2</sub>) was inoculated with thermophilic sludge (55 °C) collected from a full-scale UASB reactor processing sugarcane vinasse (São Martinho, Pradópolis, SP). The sludge was ground and directly added to the reactor to guarantee an effective contact of biomass with support material for 24 h. The excess sludge was then drained, after which the continuous feeding was initiated through applying diluted molasses with COD values of respectively 2.5, 5.0 and 10.0 g L<sup>-1</sup> at a fixed hydraulic retention time (HRT) of 2 h. This provided

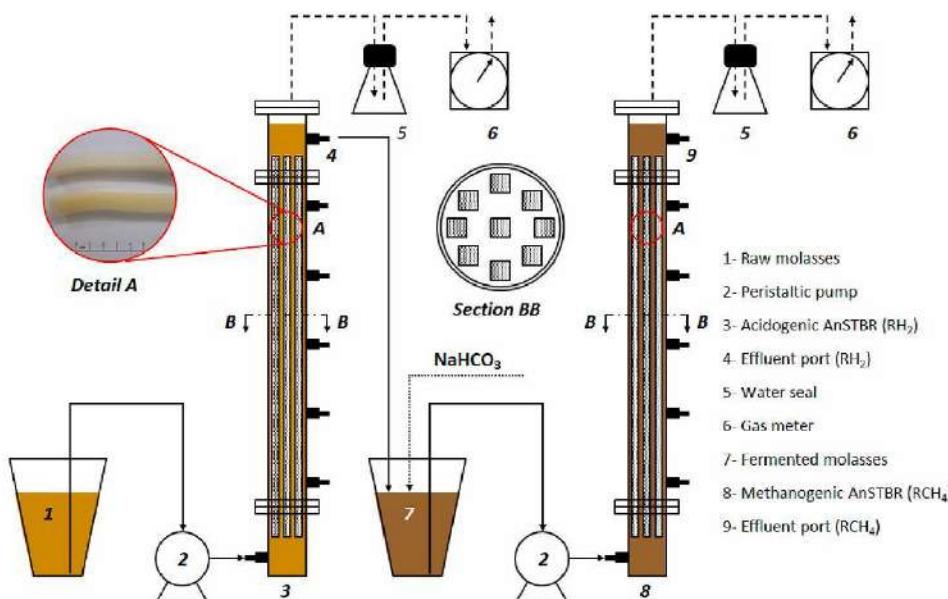


Fig. 1. Schematic diagram of the experimental set-up and details of support material distribution.

OLR values of 30, 60 and 120 kg COD m<sup>-3</sup> d<sup>-1</sup>, respectively. Details of the first 45 d of operation were previously reported elsewhere [19]. Data presented and discussed in this study refer specifically to the operation from day 168 to day 417, corresponding to 249 days of continuous operation under an OLR of 120 kg COD m<sup>-3</sup> d<sup>-1</sup> (COD = 10.0 g L<sup>-1</sup>; HRT = 2 h). Influent pH adjustment (~5.3) was not needed.

### 2.3.2. Methanogenic stage

A similar inoculation approach was applied in the startup of the methanogenic AnSTBR (RCH<sub>4</sub>) through using sludge from a full-scale UASB reactor treating poultry slaughterhouse wastewater under mesophilic condition (35 °C). The long-term monitoring of RCH<sub>4</sub> (251 d) was started with six different conditions through decreasing the HRT (240–9 h) up to the operating limit of the system, which provided a step increase in the OLR, respectively 1.0, 2.3, 4.6, 6.9, 15.8, 25.2 kg COD m<sup>-3</sup> d<sup>-1</sup>. An additional final operating phase was included by increasing the HRT to 12 h resulting in an OLR 18.5 kg COD m<sup>-3</sup> d<sup>-1</sup>, in an effort to recover the methanogenic activity. System alkalinization was provided by dosing NaHCO<sub>3</sub>, as commonly carried out in methanogenic systems [13, 14]. NaHCO<sub>3</sub> dosing was decreased throughout the operation from 1.0 to 0.2 g NaHCO<sub>3</sub> g<sup>-1</sup>COD, further reducing operational costs.

### 2.4. AD monitoring: analytical methods and performance evaluation

Reactor monitoring was based on the periodic sampling of the liquid and gas phases. Liquid phase measurements included pH, COD, CH<sub>t</sub>, intermediate (IA) and partial (PA) alkalinity and VFA for both influent and effluent streams. pH and COD, as well as parameters used in the complementary characterization of fresh molasses (BOD, TKN and SO<sub>4</sub><sup>2-</sup>; Section 2.1) were determined following protocols described in the Standard Methods for the Examination of Water and Wastewater [27]. IA and PA measurements were carried out by direct titration according to Ripley et al. [28]. CH<sub>t</sub> and VFA determinations followed the protocols described by Dubois et al. [29] and Penteado et al. [30]. Gas phase analysis was based on the monitoring of the biogas production rate and composition, respectively through gas meters (Section 2.2) and gas chromatography with a GC/TCD. Biogas composition (H<sub>2</sub>, CH<sub>4</sub> and CO<sub>2</sub>) was carried out according to Perna et al. [31].

The performance of both reactors was evaluated using the following response-variables: COD removal efficiency (ER<sub>COD</sub>; % – RH<sub>2</sub> and RCH<sub>4</sub>), CH<sub>t</sub> conversion efficiency (EC<sub>CH<sub>t</sub></sub>; % – RH<sub>2</sub>), bioH<sub>2</sub> proportion in biogas (fH<sub>2</sub>; % – RH<sub>2</sub>); volumetric hydrogen production rate (VHPR; NmL-H<sub>2</sub> L<sup>-1</sup> d<sup>-1</sup> – RH<sub>2</sub>), hydrogen yield (HY; mol H<sub>2</sub> mol<sup>-1</sup>CH<sub>t</sub> – RH<sub>2</sub>), methane proportion in biogas (fCH<sub>4</sub>; % – RCH<sub>4</sub>), volumetric methane production rate (VMPR; NmL CH<sub>4</sub> L<sup>-1</sup> d<sup>-1</sup> – RCH<sub>4</sub>), methane yield (NmL-CH<sub>4</sub> g<sup>-1</sup>COD – RCH<sub>4</sub>), and the IA/PA ratio (dimensionless - RCH<sub>4</sub>).

Monitoring data were also used to calculate COD-based mass balances (MB<sub>COD</sub>) for both reactors by comparing the measured metabolites (liquid and gas phases) with the amount of substrate available in the feed streams. Calculations were based on Eq. (1), in which the terms SPM<sub>eff</sub>, BFR and COD<sub>inf</sub> are, respectively the amount of soluble phase metabolites (CH<sub>t</sub> and organic acids), the target biogas (H<sub>2</sub> or CH<sub>4</sub>) flow rates and the amount of COD applied in the systems. All measurements were converted to COD equivalents, so that applied and removed loading values, i.e., in terms of g COD d<sup>-1</sup>, were used to determine the MB<sub>COD</sub>.

$$MB_{COD} = \frac{SPM_{eff} + BFR}{COD_{inf}} \quad (1)$$

### 2.5. Energetic potential analysis

The energetic potential of the bioH<sub>2</sub> (EP<sub>H<sub>2</sub></sub>) and methane (EP<sub>CH<sub>4</sub></sub>) produced by the two-stage AD of sugarcane molasses was calculated according to Eqs. (2) and (3), in which the terms VHPR, OLR, LHV<sub>H<sub>2</sub></sub>, MY, ER<sub>COD</sub><sup>H<sub>2</sub>, ER<sub>COD</sub><sup>CH<sub>4</sub> and LHV<sub>CH<sub>4</sub></sub> are, respectively, the volumetric</sup></sup>

hydrogen production rate (Nm<sup>3</sup>·H<sub>2</sub> m<sup>-3</sup> d<sup>-1</sup>), the organic loading rate in the acidogenic stage (kg COD m<sup>-3</sup> d<sup>-1</sup>), the lower heating value of hydrogen (MJ Nm<sup>-3</sup>H<sub>2</sub>), the methane yield (Nm<sup>3</sup>·CH<sub>4</sub> kg<sup>-1</sup>COD), the COD removal efficiency in acidogenesis (dimensionless), the COD removal efficiency in methanogenesis (dimensionless), and the lower heating value of methane (MJ Nm<sup>-3</sup>CH<sub>4</sub>). VHPR, OLR, MY, ER<sub>COD</sub><sup>H<sub>2</sub></sup> and ER<sub>COD</sub><sup>CH<sub>4</sub></sup> values were obtained experimentally in this study. In turn, LHV<sub>H<sub>2</sub></sub> and LHV<sub>CH<sub>4</sub></sub> were set, respectively as 10.71 MJ Nm<sup>-3</sup>H<sub>2</sub> and 35.72 MJ Nm<sup>-3</sup>CH<sub>4</sub> [32]. EP<sub>H<sub>2</sub></sub> and EP<sub>CH<sub>4</sub></sub> values were obtained in terms of kJ kg<sup>-1</sup>COD<sub>applied</sub>. The global energetic potential (EP<sub>global</sub>) was obtained according to Eq. (4).

$$EP_{H_2} = \frac{VHPR}{OLR} \cdot LHV_{H_2} \cdot 1000 \quad (2)$$

$$EP_{CH_4} = MY \cdot (1 - ER_{COD}^{H_2}) \cdot ER_{COD}^{CH_4} \cdot LHV_{CH_4} \cdot 1000 \quad (3)$$

$$EP_{global} = EP_{H_2} + EP_{CH_4} \quad (4)$$

### 2.6. Molecular analyses

#### 2.6.1. Sampling and DNA extraction

Biomass samplings were carried out at the same days for both reactors, corresponding to operating days 283, 312, 339, 366 and 405 for RH<sub>2</sub> and operating days 118, 149, 172, 201 and 250 for RCH<sub>4</sub>. The sampling days of RCH<sub>4</sub> corresponded to the end of each operating phase with different HRT and OLR (Section 2.3.2). Biomass sampling was based on pooled composed specimens, after centrifugation. Further biomass removal from PU foams and DNA extraction were carried out following the procedures described elsewhere [19]. Briefly, for metagenomics the phenol:chloroform extraction method was employed, while for qPCR analyses the PowerSoil® DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) was preferred. DNA quality was assessed through a ND-2000 spectrophotometer (Nanodrop Inc., Wilmington, DE) and agarose gel electrophoresis.

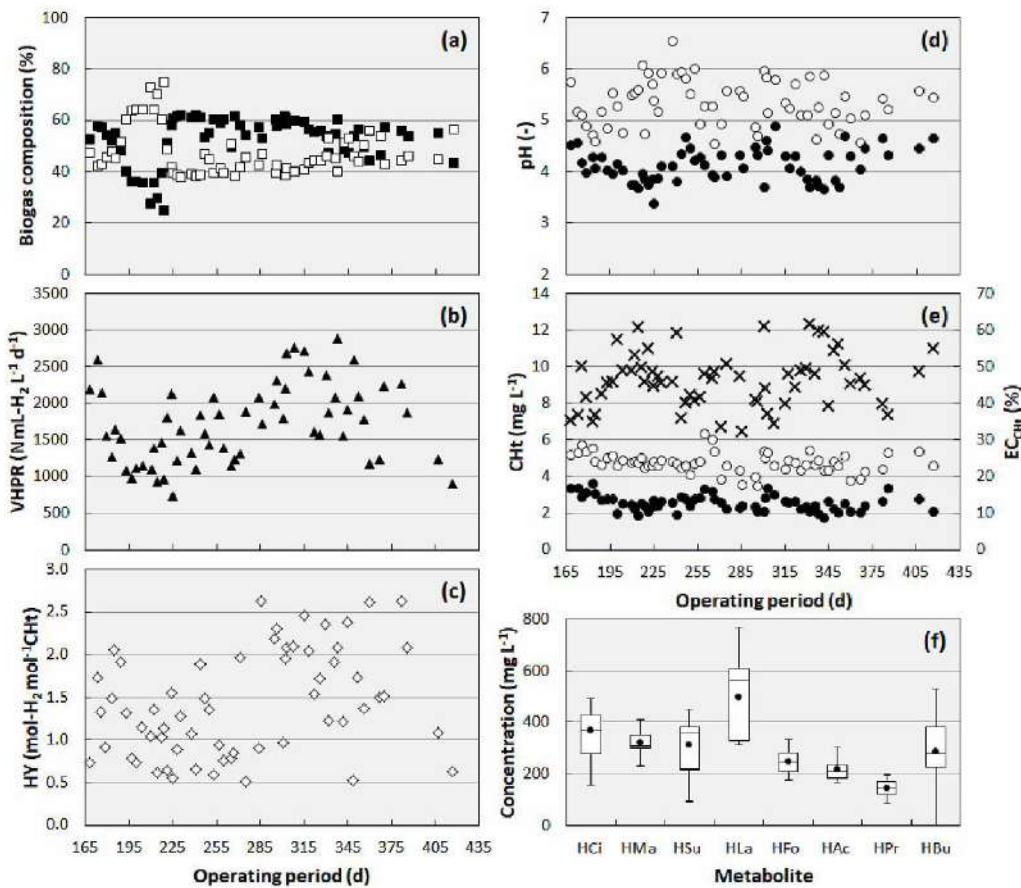
#### 2.6.2. Metagenomic and qPCR analyses

The metagenomic analysis involved 16 S rRNA amplicons using the primer set described in Klindworth et al. [33], containing adapter overhangs, according to manufacturer's recommendations (Illumina manual). The sequencing was performed as a service from the Functional Genomics Center (ESALQ/USP, Piracicaba, Brazil), using an Illumina MiSeq platform, according to the manufacturer's guidelines for paired end samples using the MiSeq v3 kits. Sequences were analyzed following the procedures reported elsewhere [19]. Metabolic functions were inferred using the database provided elsewhere [34] after proper annotation. Canonical correspondence analysis (CCA) was further used to correlate community structure and the chemical variables [35]. For the CCA, a cutoff of 0.5% abundance was used for the genera. Additionally, qPCR was used to quantify Fe-hydrogenase and methanogenic Archaea (McrA) genes. Amplification used primers HydA-F and HydA-R for Fe-hydrogenase [36] and primers ME\_1F and ME\_3 R for McrA [37]. Further procedure details are presented elsewhere [19].

## 3. Results and discussion

### 3.1. Acidogenic stage: long-term bioH<sub>2</sub> production

The acidogenic reactor was monitored for 249 days under a constant OLR (120 kg COD m<sup>-3</sup> d<sup>-1</sup>). Overall performance data regarding both gas and liquid phases are presented in Fig. 2. Complementary performance data for RH<sub>2</sub> are presented in the Supplementary data section. Continuous bioH<sub>2</sub> production levels were observed within the entire operating period of RH<sub>2</sub>, with an average fH<sub>2</sub> of ~50% (Fig. 2a). No methane production was identified in RH<sub>2</sub>, indicating that the imposed operating conditions, i.e., high ORL/low HRT suppressed the activity of



**Fig. 2.** Overall performance of the acidogenic stage (RH<sub>2</sub>): temporal profiles for the (a) biogas composition (■- H<sub>2</sub>, □- CO<sub>2</sub>), (b) volumetric hydrogen production rate (VHPR, ▲), (c) hydrogen yield (HY, ◇), (d) pH (○- influent, ●- effluent) and (e) carbohydrate conversion efficiency (○- influent CHt concentration, ●- effluent CHt concentration, × - EC<sub>CHt</sub>); and, (f) soluble phase metabolite (SPM) concentrations in fermented molasses (●- average values). Legend (SPM): HCl-citric acid, HMa-malic acid, HSu-succinic acid, HLa-lactic acid, HFo-formic acid, HAc-acetic acid, HPr-propionic acid, HBu-butyric acid.

methanogens. The average VHPR and HY values were respectively,  $\sim 1700 \text{ NmL-H}_2 \text{ L}^{-1} \text{ d}^{-1}$  (Fig. 2b) and  $\sim 1.4 \text{ mol-H}_2 \text{ mol}^{-1} \text{ CHt}$  (Fig. 2c). Despite the unstable pattern (coefficient of variation of 30% for the VHPR), the hydrogenogenic activity was effectively maintained in the system, with genes encoding for Fe-hydrogenase present during the operating period (Table 1), irrespective of the low effluent pH values ( $< 5.0$ ; Fig. 2d). The literature indicates that pH values higher than 5.0 [16,38] are required to achieve efficient bioH<sub>2</sub>-producing conditions in thermophilic acidogenic systems, fed with vinasse. However, discrepant patterns were observed for molasses, both in this study and in recent related investigations using PU-filled AnSTBR systems, still resulting in bioH<sub>2</sub> production. Similar performance was previously achieved in the operation of RH<sub>2</sub> under the OLR of  $120 \text{ kg COD m}^{-3} \text{ d}^{-1}$  (days 27–45 [19]), indicating an average VHPR of  $2041 \text{ NmL-H}_2 \text{ L}^{-1} \text{ d}^{-1}$  and low effluent pH values of (4.3). Oliveira et al. [18] reported similar VHPR ( $89 \text{ NmL-H}_2 \text{ L}^{-1} \text{ h}^{-1}$  or  $2136 \text{ NmL-H}_2 \text{ L}^{-1} \text{ d}^{-1}$ ) and HY ( $1.4 \text{ mol-H}_2 \text{ mol}^{-1} \text{ CHt}$ ) values even at lower pH values (3.8), applying an OLR of  $60 \text{ kg COD m}^{-3} \text{ d}^{-1}$  in an AnSTBR inoculated with fermented vinasse.

Similar CHt conversion patterns were also observed in all compared systems, i.e., 46.2% (this study; Figs. 2e), 40.4% [18] and 55.0% [19]. The relatively low values may be explained by the low HRT values applied (2–4 h). Although bioH<sub>2</sub> production in expanded granular sludge-bed reactors has been described [17], differences regarding the dynamics of the reactors as well as the operating conditions (e.g. mesophilic temperature) hinder an effective comparison with this study.

Performance data from RH<sub>2</sub> can be compared with acidogenesis of sugarcane vinasse, by using fixed-film systems and thermophilic conditions ( $55^\circ\text{C}$ ). Packed-bed vinasse-fed reactors subjected to OLR of  $72\text{--}108 \text{ kg COD m}^{-3} \text{ d}^{-1}$  [22,38,39] reported lower VHPR values ( $391\text{--}1604 \text{ NmL-H}_2 \text{ L}^{-1} \text{ d}^{-1}$ ). Moreover, the cessation of the hydrogenogenic activity was observed within 30–60 days when proper operating strategies were not applied, such as the maintenance of pH higher than 5.0 and the removal of excess biomass [38]. The low porosity of such reactor configuration has been pointed as a limiting factor to attain efficient bioH<sub>2</sub> production levels. The application of the AnSTBR in vinasse acidogenesis markedly improved the hydrogenogenic activity [16], leading to a maximum experimental VHPR of  $2074 \text{ NmL-H}_2 \text{ L}^{-1} \text{ d}^{-1}$  (average value; OLR =  $87.5 \text{ kg COD m}^{-3} \text{ d}^{-1}$ ) similar to the ones obtained for molasses. However, the strict control of fermentation pH is always required. Overall, results obtained in this study and in the literature indicate that the less complex composition of molasses favors bioH<sub>2</sub>-production under harsh conditions, which limits the hydrogenogenic activity in vinasse-fed systems. Differences regarding the source of inoculum may also explain the observed performance, as

**Table 1**  
Gene copy numbers for Fe-hydrogenase samples from RH<sub>2</sub> during the operating period.

Operating period (d)	283	312	339	366	405
Log n. copies of genes encoding for Fe-hydrogenase ng <sup>-1</sup> DNA	8.2	8.9	8.6	8.6	9.0

natural fermentation is usually used to provide acidogenic populations in vinasse-fed reactors [16,22,38,39]. The results obtained in molasses-fed thermophilic AnSTBR systems point to an efficient combination of substrate-reactor type-operating condition, opening further possibilities to improve system performance, mainly targeting to increase biogas production.

The primary metabolites detected in RH<sub>2</sub> included acetate (HAc; ~220 mg L<sup>-1</sup>), butyrate (HBu; ~300 mg L<sup>-1</sup>), formate (HFo; ~250 mg L<sup>-1</sup>), propionate (HPr; ~150 mg L<sup>-1</sup>) and lactate (HLa; ~500 mg L<sup>-1</sup>) (Fig. 2f). BioH<sub>2</sub> production in RH<sub>2</sub> was most likely associated with the acetic- and butyric-type fermentation pathways (Reactions 1 and 2, respectively [40]), which corresponds to the most desired

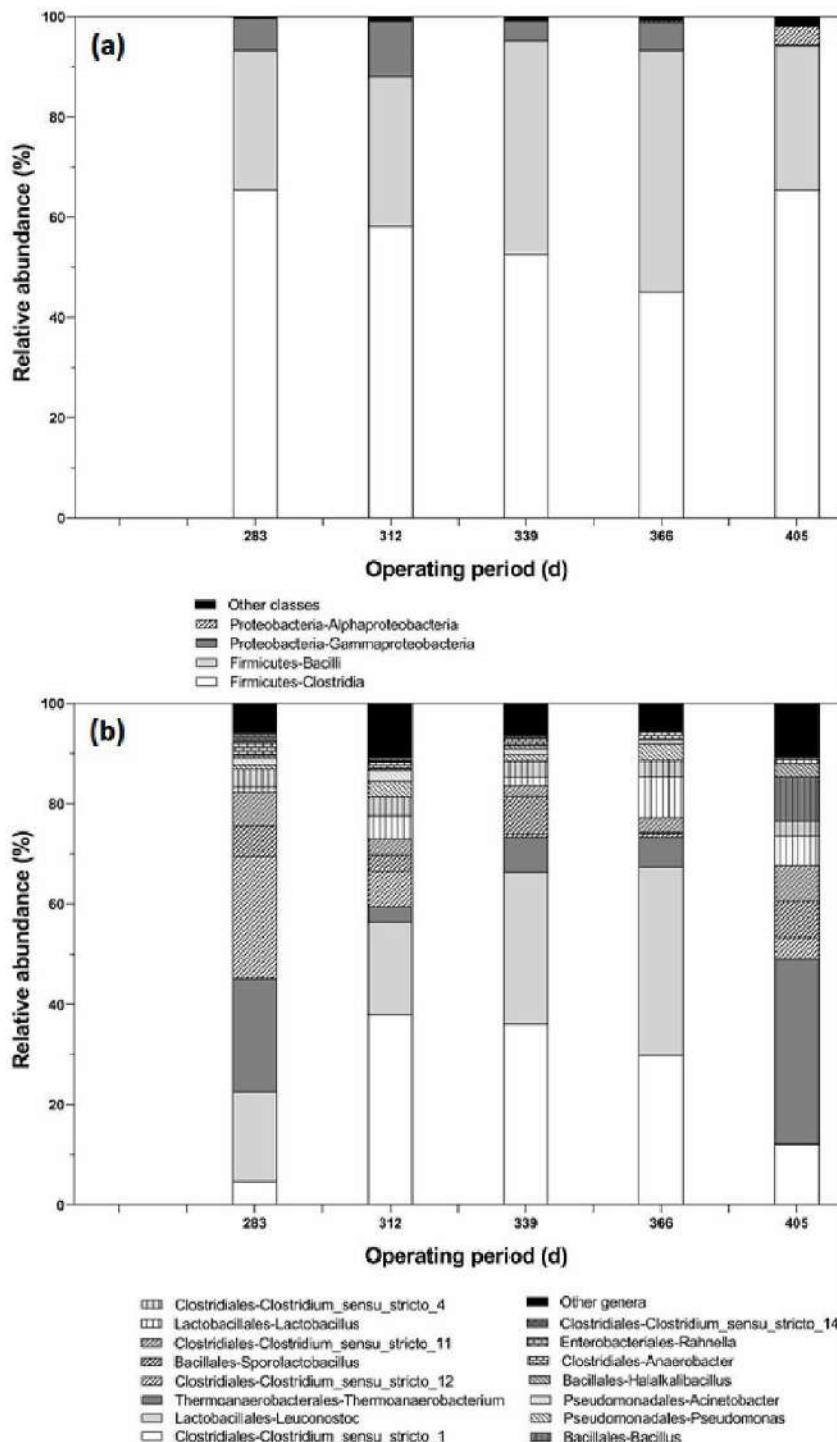
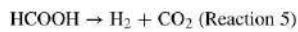
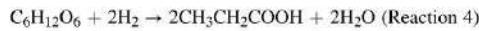
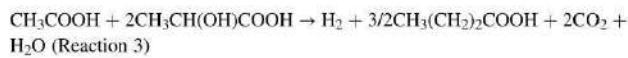
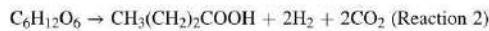
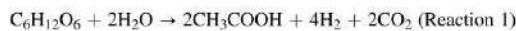


Fig. 3. Taxonomic affiliation of the sequences derived from RH<sub>2</sub> during the operating period: (a) Phylum and Class levels, (b) Order and Genus levels.

bioH<sub>2</sub>-producing mechanisms in acidogenic systems. In particular, metabolite concentrations in COD-equivalents (HAc = 235 mg COD L<sup>-1</sup> and HBu = 546 mg COD L<sup>-1</sup>) show the predominance of the butyric-type fermentation in the system, and the relatively high HLa concentrations show alternative bioH<sub>2</sub>-producing pathways, such as the co-fermentation of HLa and HAc (Reaction 3 [41]) which also generates HBu. The co-fermentation of HLa and HAc has been previously identified in molasses [18] and vinasse [16] thermophilic fermentation, showing that this pathway was relevant to bioH<sub>2</sub> production in RH<sub>2</sub>, considering the similar operating conditions. The relatively high HPr concentration (226 mg COD L<sup>-1</sup>) indicates also a propionic activity (Reaction 4 [40]) equivalent to the acetic one. This pattern suggests the establishment of organic overloading conditions, which may have decreased the hydrogenogenic activity to control the H<sub>2</sub> partial pressure within the system. HFo accumulation is a complementary evidence of the organic overloading, as the production of bioH<sub>2</sub> from this particular substrate depends on its decomposition (Reaction 5 [42]), i.e., bioH<sub>2</sub> accumulation most likely hindered this pathway. Despite those unexpected pathways, continuous bioH<sub>2</sub> production was successfully achieved in RH<sub>2</sub>, which indicates that the application of lower OLR levels increases the bioH<sub>2</sub> production potential.



### 3.1.1. Characterization of the microbial community by amplicon metagenomics of the 16 S rDNA in RH<sub>2</sub>

A total of 1,875,277 high quality sequences of 1,899,775 raw paired-end reads were obtained and clustered into operational taxonomic units (OTU) for annotation, corresponding to: 460,920 sequences (day 283), 311,644 sequences (day 312), 375,274 sequences (day 339), 300,769 (day 366) and 426,670 sequences (day 405). Taxonomic data showed that *Firmicutes* was the most dominant group (88–95%) at the Phylum level throughout the operating period with selection of *Clostridia* and *Bacilli* Classes, followed by *Alpha-* and *Gamma-Proteobacteria* (5–11%) (Fig. 3a). *Firmicutes* includes most of the bioH<sub>2</sub>-producing microbes, which can utilize numerous compounds, e.g. carbohydrates and proteins [43,44], for their growth.

At the day 283, sequences related to bioH<sub>2</sub>-producing bacteria of the *Clostridia* class, such as *Thermoanaerobacterium* (22%) and *Clostridium sensu stricto* (average sum of five genera of 41%, with *Clostridium sensu stricto* 12 adding up to 24%), were the most representative followed the *Bacilli* class, such as *Leuconostoc* (18%) and *Sporolactobacillus* (6%). *Thermoanaerobacterium* was observed in vinasse [25] and molasses-fed [18,19,26] thermophilic reactors operated for bioH<sub>2</sub> production. *T. thermosaccharolyticum*, for example, is able to produce hydrogen via butyrate-acetate fermentation under thermophilic condition [45]. *Clostridium sensu stricto* 12 has been identified in a bioH<sub>2</sub>-producing molasses-fed thermophilic reactor [19]. Lactic acid bacteria (LAB) belonging to the *Leuconostocaceae* family were also positively related with bioH<sub>2</sub> production most probably via co-fermentation of lactate and acetate [46]. Although *Sporolactobacillus* was reported in acidogenic reactor before [47], the authors did not discuss its function in bioH<sub>2</sub> production. At the days 312, 339 and 366, selection of *Clostridium sensu stricto* 1 (average of 35%) and *Leuconostoc* (average of 29%) was observed (Fig. 3b). In this period, VHPR and HY reached the highest values (Fig. 2b and c). An increase in the relative abundance of *Thermoanaerobacterium* (37%) and decrease in *Clostridium sensu stricto* 1

(12%) as well as in *Leuconostoc* (0.24%) was observed at the day 405 (Fig. 3b), when decreasing patterns in both the VHPR and HY were also measured (Fig. 2b and c). These findings suggest a positive correlation of *Clostridium sensu stricto* 1 and LAB with bioH<sub>2</sub> production under thermophilic temperature. Similar results were also reported in bioH<sub>2</sub>-producing systems fed with glucose [44] and beet molasses [43] operated at mesophilic temperature.

CCA analysis was applied to better understand the correlation between the microbiota and the other parameters monitored [35]. Fig. 4 shows that among the present microbiota throughout the operating period, *Thermoanaerobacterium* had a positive correlation with the degradation of organic matter (COD) and with bioH<sub>2</sub> production; *Clostridium sensu stricto* 12 and 14 were positively correlated with production of acids while *Clostridium sensu stricto* 1 and *Leuconostoc* and other LAB (*Lactobacillus* and *Lactococcus*) had a positive correlation with bioH<sub>2</sub> production and pH. These findings, together with the identification of co-fermentation of HLa and HAc in the reactor, strongly suggest a positive interaction of *Clostridium sensu stricto* 1 and LAB, more specifically *Leuconostoc*, to bioH<sub>2</sub> production in RH<sub>2</sub>.

Functional groups were inferred from the taxonomic description using a manually curated database as previously presented elsewhere [34]. Fermentation was predominant during operation of the reactor followed by unknown metabolic functions. Complementary metabolic function data are presented in the Supplementary data section. Similar results were obtained in previous study on the context of the thermophilic fermentation of molasses [19], showing that operating conditions strongly selected fermentation with microorganisms involved in bioH<sub>2</sub> production, and that unknown metabolic function is a gap to overcome. Some strategies proposed in Vilela et al. [19], such as increased cultivation efforts or shotgun metagenomic approaches, could help to overcome this gap, by clarifying some aspects of the multi-process microbiota participating in substrate conversion, providing data to improve the operation of the system.

### 3.2. Methanogenic stage: long-term system stability

The methanogenic reactor was continuously fed with fermented molasses for 251 days, based on the increase of the OLR (1.0–25.2 kg COD m<sup>-3</sup> d<sup>-1</sup>) by decreasing the HRT (240–12 h). Performance details (temporal profiles) of the methanogenic stage are presented in Fig. 5,

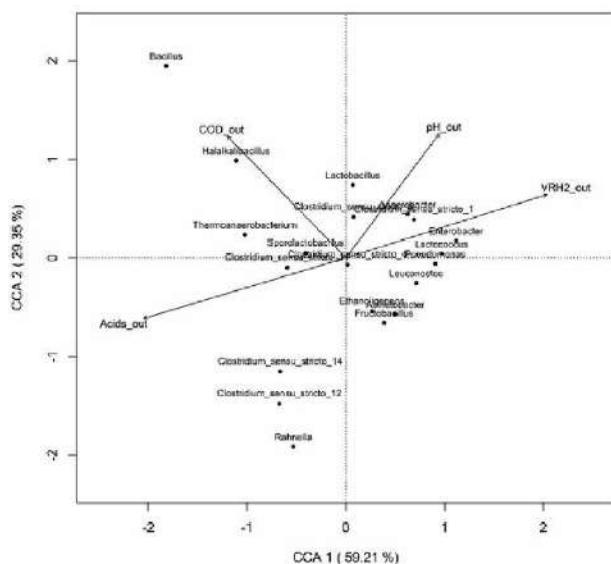
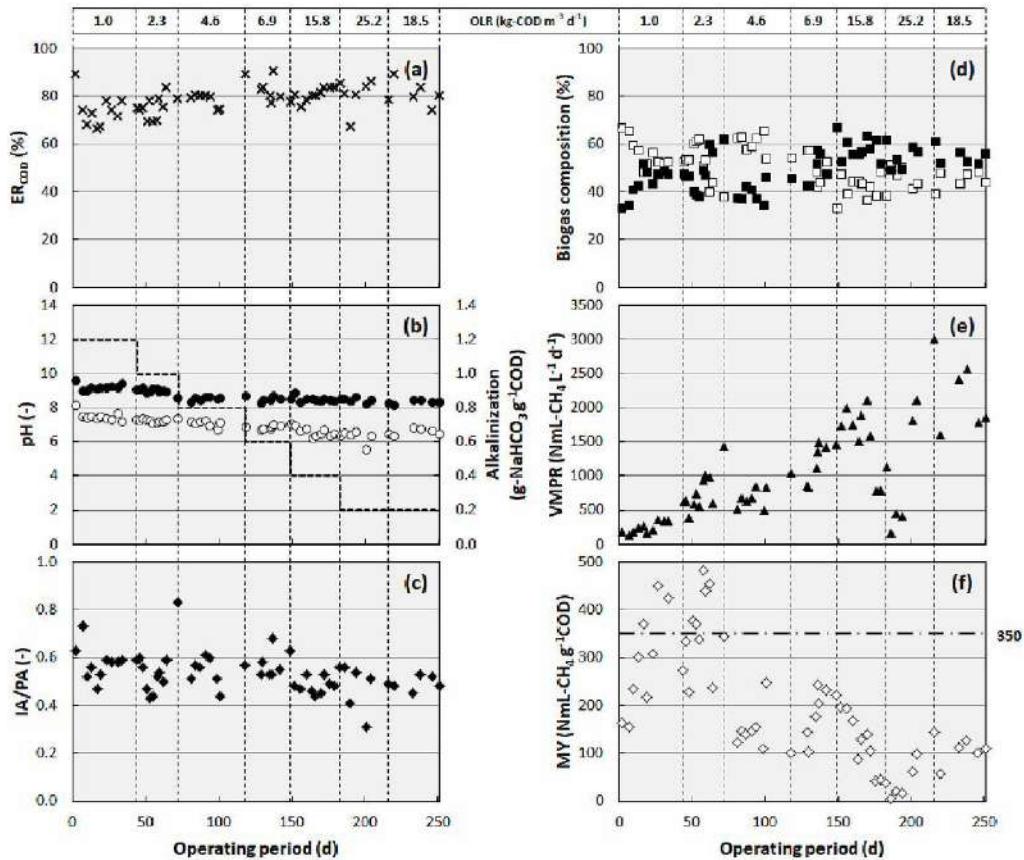


Fig. 4. Canonical correspondence analysis (CCA) carried out with physical-chemical and microbial data obtained from RH<sub>2</sub>.



**Fig. 5.** Overall performance of the methanogenic stage (RCH<sub>4</sub>): temporal profiles for the (a) COD removal efficiency (ER<sub>COD</sub>,  $\times$ ), (b) pH ( $\circ$ - influent,  $\bullet$ - effluent), (c) intermediate (IA) to partial (PA) alkalinity ratio (IA/PA,  $\blacklozenge$ ), (d) biogas composition ( $\blacksquare$ - CH<sub>4</sub>,  $\square$ - CO<sub>2</sub>), (e) volumetric methane production rate (VMMPR,  $\blacktriangle$ ) and (f) methane yield (MY,  $\lozenge$ ).

whilst complementary data are presented in the Supplementary data section. High ER<sub>COD</sub> levels (>70%; Fig. 5a) were observed in all operating conditions, regardless of the high OLR and decreasing doses of NaHCO<sub>3</sub> (1.2–0.2 g NaHCO<sub>3</sub> g<sup>-1</sup> COD; Fig. 5b). The stepwise decrease in the alkalization via NaHCO<sub>3</sub> dosing was implemented in an effort to obtain lower effluent pH levels, which reached values higher than 9.0 in the first two operating conditions (Fig. 5b). This strategy has been similarly applied in vinasse-fed methanogenic systems [14] aiming to prevent inhibitory effects of pH values higher than 8.3 [48]; however, system performance was negatively impacted in that case. In this case, COD removal was not impacted by either the reduction in the supply of NaHCO<sub>3</sub> or the high effluent pH values, which remained approximately between 8.3 and 8.5 (Fig. 5b) until the end of the operating period. System stability was established within an IA/PA ratio usually ranging between 0.5 and 0.6 (Fig. 5c), irrespective of the OLR. This particular result proves the suitability to achieve robust operating conditions at IA/PA values considerably higher than the reference level for wastewater, i.e., 0.2–0.3, as previously observed in the anaerobic processing of sugarcane vinasse [14,24].

Regarding methane production, fCH<sub>4</sub> values ranged approximately between 50 and 60% during the entire operating period (Fig. 5d). However, marked discrepant patterns were observed for the VMMPR (Fig. 5e) and MY (Fig. 5f) as the OLR was increased. VMMPR values increased with an increase in the OLR (Fig. 5e) except for the OLR of 25.2 kg COD m<sup>-3</sup> d<sup>-1</sup>), whilst the MY decreased at OLR values exceeding 2.3 kg COD m<sup>-3</sup> d<sup>-1</sup> (Fig. 5f). This discrepancy may be strictly associated with the conversion efficiency of organic matter by methane-producing pathways. Increasing VMMPR values most likely resulted

from the higher substrate availability at higher OLR, which may not be directly associated with an effective methanogenic activity. Furthermore, the drop observed in MY indicates unfavorable conditions for methanogens at higher OLR. However, the loss observed in methane production did not impact system stability, as indicated by the maintenance of high ER<sub>COD</sub> values (Fig. 5a), even at high OLR.

The simultaneous observation of low MY and high ER<sub>COD</sub> can be explained by a shift in conversion pathways, as substrate uptake was most likely diverted to cell production at OLR values higher than 2.3–4.6 kg COD m<sup>-3</sup> d<sup>-1</sup>. Using mesophilic sludge in the inoculation of the system (Section 2.3.2) supports this hypothesis, as the biomass (primarily methanogenic populations) was not acclimatized to the thermophilic conditions when high OLR (>2.3 kg COD m<sup>-3</sup> d<sup>-1</sup>) were applied. Döll and Foresti [49] reported similar limitations using the same source of inoculum when comparing mesophilic and thermophilic sequencing-batch reactors in the treatment of sugarcane vinasse. Increasing the temperature from 35 to 55 °C severely impacted the performance of the thermophilic system, which limited the increase of the OLR to 5.2–5.7 kg COD m<sup>-3</sup> d<sup>-1</sup> (while 36.0 kg COD m<sup>-3</sup> d<sup>-1</sup> was achieved in the mesophilic reactor), and required high bicarbonate dosing levels (0.6 vs. 0.2 g HCO<sub>3</sub> g<sup>-1</sup> COD). Sharp decreases in the methanogenic activity were also previously reported by van Lier et al. [50] when increasing the temperature (38–55 °C) in UASB reactors treating VFA mixtures. In this case, 60 days were required to establish an efficient CH<sub>4</sub> production after stepwise increase of the temperature. Conversely, no specific procedures to adapt the biomass to high temperatures were carried out in RCH<sub>4</sub> prior to increasing the OLR. Theoretically, additional pathways, such as sulfate reduction through the

complete oxidation of organic matter, could also explain the high  $ER_{COD}$  values under impaired methane-producing conditions. However, the low sulfate concentrations detected in molasses ( $CODs/SO_4^{2-} = 112.6$ ; Section 2.1) indicate a negligible contribution of sulfidogenesis in RCH<sub>4</sub>.

The breakdown of metabolites (Fig. 6) in biodigested molasses indicated an accumulation of HLa, HAc and, to a lower extent, HPr at high OLR values ( $>6.9 \text{ kg COD m}^{-3} \text{ d}^{-1}$ ; Fig. 6c), which provides insights on the prevailing dynamics of organic matter conversion. HAc accumulation is an immediate evidence of an unfavorable environment to acetoclastic methanogens in these operational conditions, which corroborates the decreasing MY values. Ferraz Jr. et al. [13] and Fuess et al. [14] also observed the accumulation of HAc and HPr at high OLR ( $25-30 \text{ kg COD m}^{-3} \text{ d}^{-1}$ ) in thermophilic AnSTBR and UASB systems, indicating the establishment of an unsuitable environment to

acetoclastic methanogens. In turn, HPr and HLa accumulation in RCH<sub>4</sub> shows an inefficient syntrophic activity, as a consequence of higher residual HAc levels. Particularly the high HLa concentrations ( $>300 \text{ mg L}^{-1}$  in most of sampling points; Fig. 6c) indicate an effective acidogenic activity in RCH<sub>4</sub>, which could be associated with biomass production. The negligible volatile suspended solids concentration in biodigested molasses (data not shown) suggests enhanced biomass retention within RCH<sub>4</sub>, which explains the maintenance of high COD removal, regardless of impaired methane-producing activities.

The type of substrate used in this study has favored biomass production, as the high biodegradable content of fermented molasses, and the high levels of residual CHt concentrations ( $2.54 \text{ g L}^{-1}$ ) stimulated the growth of specific microbial populations, including fermentative ones (Section 3.2.1). Both the MY and  $ER_{COD}$  were correlated in the thermophilic biodigestion of fermented sugarcane vinasse [13,14,24], i.e., MY increased as improvements were observed in COD removal. This difference resulted from the low residual CHt in fermented vinasse ( $0.94 \text{ g L}^{-1}$  [13]), stimulating acidogenesis (and the subsequent "sequestration" of COD as retained biomass) less effectively compared to this study.

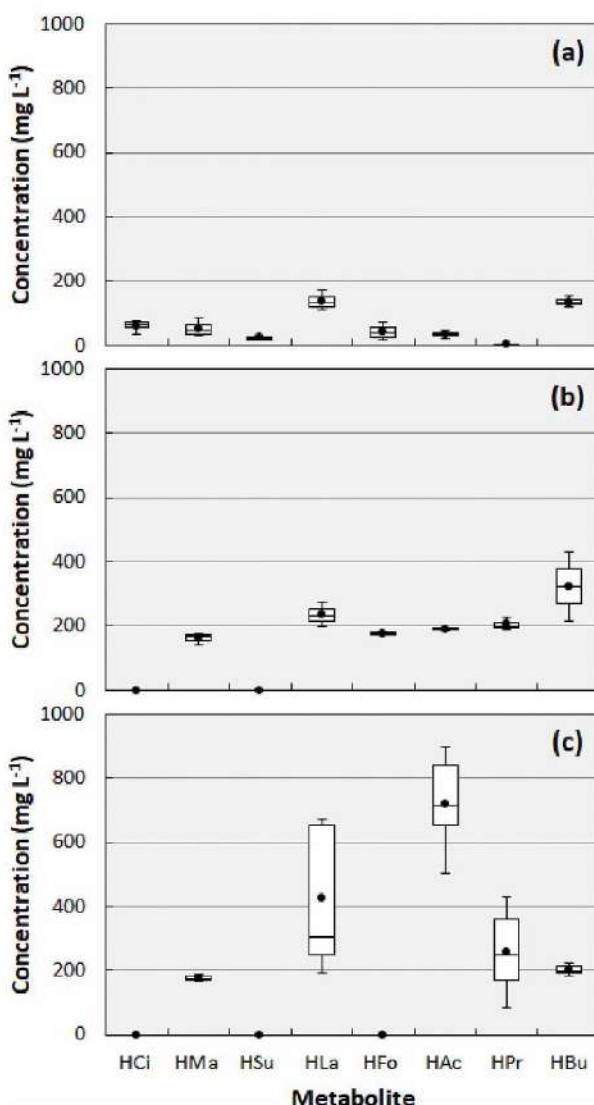
### 3.2.1. Characterization of the microbial community by amplicon metagenomics of the 16 S rDNA in RCH<sub>4</sub>

From a total of 2,332,525 raw paired-ended reads obtained from the 16 S rDNA sequencing of samples collected from RCH<sub>4</sub> and its inoculum, about 9.11% were discarded after quality control, providing 2,120,020 high quality sequences, which overlapped and were clustered into OTU for annotation. These corresponded to 207,481 sequences of the inoculum, 430,889 ( $4.6 \text{ kg COD m}^{-3} \text{ d}^{-1}$ ), 397,583 ( $6.9 \text{ kg COD m}^{-3} \text{ d}^{-1}$ ), 402,347 ( $15.8 \text{ kg COD m}^{-3} \text{ d}^{-1}$ ), 240,036 ( $25.2 \text{ kg COD m}^{-3} \text{ d}^{-1}$ ) and 441,684 ( $18.5 \text{ kg COD m}^{-3} \text{ d}^{-1}$ ).

The mesophilic sludge collected from the UASB reactor treating poultry slaughterhouse wastewater used as inoculum in RCH<sub>4</sub> showed a diverse composition. At the Class level, *Clostridia* (38.6%), *Aerolineae* (11.7%) and *Thermotogae* (7.4%) were the most abundant groups, followed in minor frequency by *Bacilli* (4.4%), *Negativicutes* (4.6%), uncultured *Armatimonadetes* (4.36%) and *Gamma-proteobacteria* (2.3%) (Fig. 7a). The microbial community of RCH<sub>4</sub> was analyzed at the end of each operating phase, corresponding to a different OLR. Overall, composition and abundance of the classes were maintained and did not differ from the inoculum, except the increase in abundance of *Bacteroidia* throughout the operating period. At the end of the operating period (OLR of  $18.5 \text{ kg COD m}^{-3} \text{ d}^{-1}$ ), *Bacteroidia* and *Thermotogae* increased in relative abundance to 27% and 21.6% respectively, as well as *Bacilli* (9.7%), *Alpha-proteobacteria* (5.3%) and *Coprotrothermobacteria* (5.3%) (Fig. 7a). *Clostridia*, *Bacteriodia*, *Aerolineae* and *Thermotogae* are the core bacterial groups in both one and two-stage methanogenic reactors, because their members are involved in the uptake of carbohydrates and fatty acids, as well as in syntrophic acetate and butyrate oxidation [51–56]. The classes found in minor frequency are also reported in anaerobic reactors, as they comprise of fermentative members, as well as HAc and HBu oxidizers.

At the genera level, the abundance of some groups found in the inoculum diminished in the reactor (Fig. 7b), and most of them increased in relation to rarer members. The abundance of each genus varied between phases without a defined pattern (Fig. 7b). Genera *Thermanaerothrix* (*Aerolineae*), *TTA-B61* and *Cellulosibacter* (*Clostridia*) were counter selected during the operation of the reactor, with a sum average of 10%. *Cellulosibacter* comprises cellulolytic bacteria with only one species isolated so far [57] and *T. daxensis*, the only species described of *Thermanaerothrix* genus, ferments sugars and organic acids to HLa, HAc and CO<sub>2</sub> [58]. Even though the abundance of these genera decreased throughout the operating period, they may have contributed to fermentation in RCH<sub>4</sub>.

The high levels of residual CHt concentrations ( $2.54 \text{ g L}^{-1}$ ) in the fermented molasses selected fermentative microorganisms (sum average



**Fig. 6.** Soluble phase metabolite concentrations in biodigested molasses according to the operating phases: (a) phases I-II ( $OLR = 1.0-2.3 \text{ kg COD m}^{-3} \text{ d}^{-1}$ ), (b) phase III ( $OLR = 4.6 \text{ kg COD m}^{-3} \text{ d}^{-1}$ ) and (c) phases IV-VII ( $OLR = 6.9-25.2 \text{ kg COD m}^{-3} \text{ d}^{-1}$ ) (● - average values). Legend: HCl-citric acid, HMa-malic acid, HSu-succinic acid, HLa-lactic acid, HFo-formic acid, HAc-acetic acid, HPr-propionic acid, HBu-butyric acid.

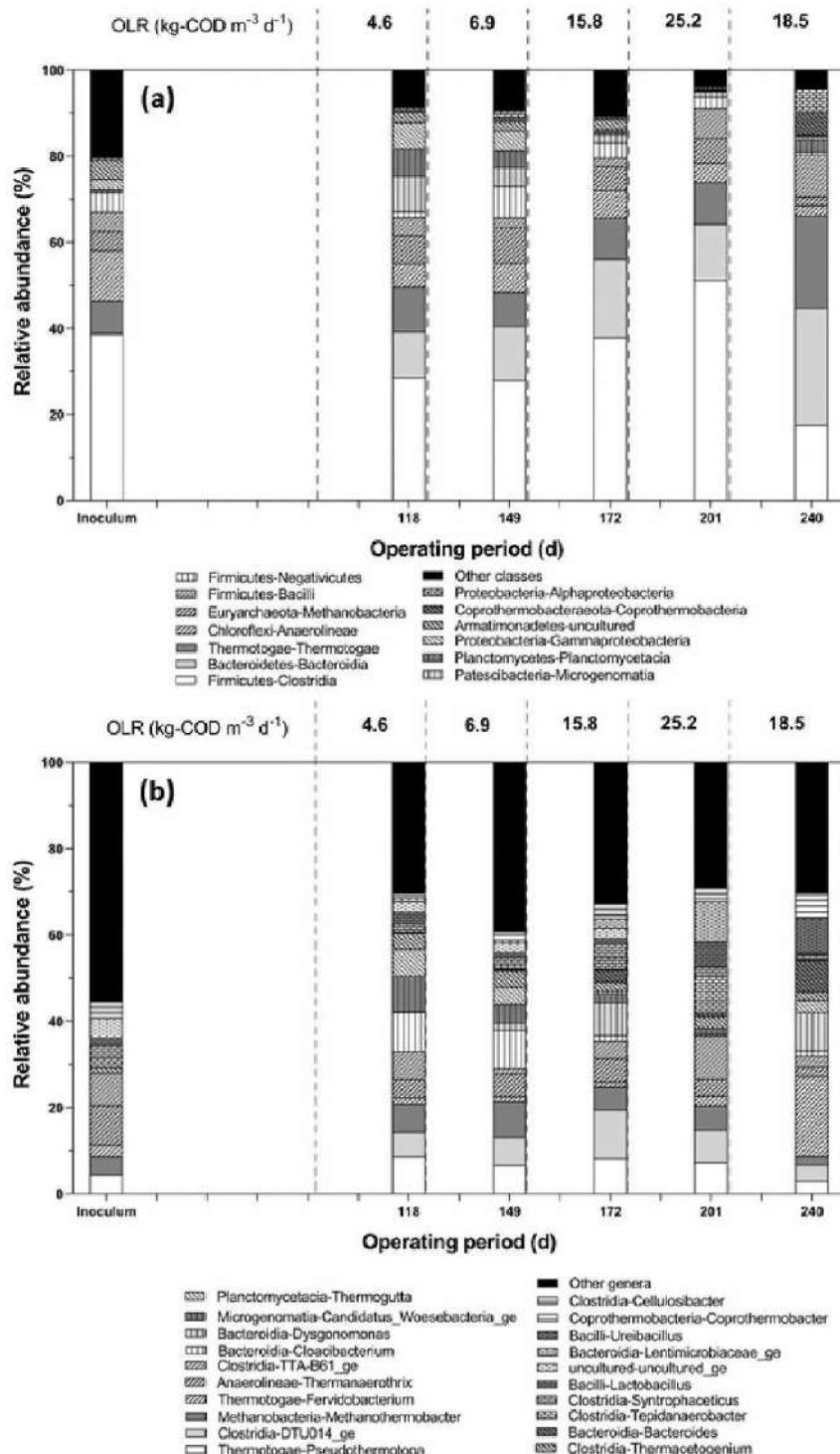


Fig. 7. Taxonomic affiliation of the sequences derived from RCH<sub>4</sub> during the operating period: (a) Phylum and Class levels, (b) Order and Genus levels.

of 28.3% of overall community) in RCH<sub>4</sub>, being the community composed by several genera, such as *Lactobacillus*, *Cloacibacterium*, *Dysgonomonas*, *Bacteroides*, *Pseudothermotoga*, *Fervidobacterium*, *Coprothermobacter* and *Thermogutta* (Fig. 7b). The *Lactobacillus* genus is a LAB group, observed with frequencies ranging from 1.3 to 5.6%, with the highest value at the highest OLR (25.2 kg COD m<sup>-3</sup> d<sup>-1</sup>). The genera

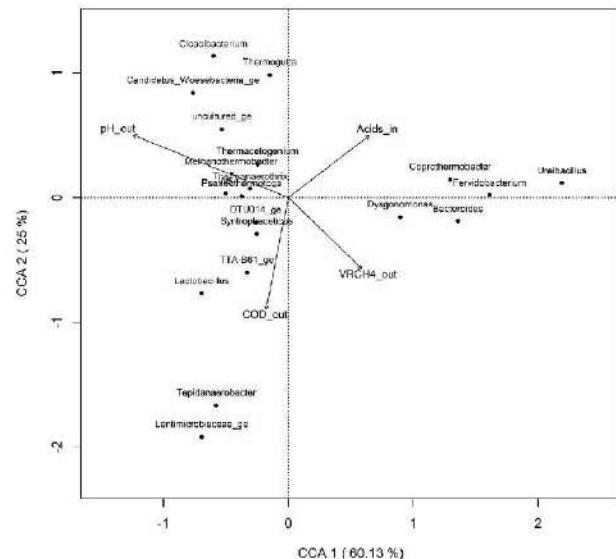
*Cloacibacterium*, *Dysgonomonas*, *Bacteroides* and *Lentimicrobiaceae*, which belong to the *Bacteroidia* class, were present in the inoculum at very low frequencies (<1%), being selected throughout the operating period with abundance of 1%–9% (Fig. 7b). *Cloacibacterium*, *Dysgonomonas* and *Bacteroides* have been detected in human gut microbiota, as well as in thermophilic and mesophilic anaerobic reactors, and are known for their

capability to ferment carbohydrates [59,60]. *Pseudothermotoga* and *Fervidobacterium* (*Thermotoga* Class) genera were found in highest abundances in different operating phases. *Pseudothermotoga* frequencies varied from 6% to 8% in all phases, with a minimum of 3% at the final phase, whilst frequencies for *Fervidobacterium* ranged between 1 and 2% in all phases, peaking at 18% at the same final phase. Species of these genera ferment carbohydrates to produce HAc, CO<sub>2</sub>, H<sub>2</sub>, HLa, ethanol, alanine, and α-aminobutyrate [61]. *Coprothermobacter* was selected at the initial OLR with 6.8% of frequency. Members of this genus are known by their capability to ferment proteins and have been reported in methanogenic reactors degrading sugarcane stillage under thermophilic conditions [55,56,62]. Although protein concentrations were not measured in fermented molasses, they could be present in this kind of effluent as reported elsewhere [51] and thus fermented in RCH<sub>4</sub> to produce amino acids, organic acids and H<sub>2</sub>.

The syntrophic genera, *Thermacetogenium*, *Tepidanaerobacter* and *Syntrophaceticus* were detected with a sum average of 7.8% and varied in relative abundance among phases. *Thermacetogenium* had its peak at initial phases (3.8%), *Tepidanaerobacter* at the highest OLR (8.5%) and *Syntrophaceticus* at the second highest OLR (3.1%) (Fig. 7b). All these genera belong to the *Clostridia* Class that oxidizes H<sub>Bu</sub>, H<sub>La</sub> and H<sub>Ac</sub> in co-culture with hydrogenotrophic methanogens [56,63–68].

*Methanothermobacter* was the main methanogen in the community, comprising of thermophile hydrogenotrophic archaea, that remained present in considerable abundance in the inoculum and throughout the entire operating period (5.35–8.30%), particularly in the early phases, and only decayed in the last phase to approximately 2% (Fig. 7b). *Methanothermobacter* corresponded to 95–96% of the Archaea Domain abundance, followed by uncultured *Methanobacteriaceae* (1–3%) and in less abundance the hydrogenotrophic methanogen *Methanopyrus* (0.1–0.2%), the sulfur metabolizing *Sulfurisphaera* (0.1–0.4%), *Sulfovobococcus* (0.2–0.3%) and *Caldivirga* (0.1–0.2%). Previous studies showed that hydrogenotrophic methanogenesis via syntrophic pathway and with predominance of *Methanothermobacter* and *Methanomicrobiales* Class, is important in thermophilic systems fed with sugarcane vinasse [53,55,56], as well as in mesophilic system fed with fermented beet molasses [51]. Hydrogenotrophic methanogenesis, carried out by *Methanothermobacter*, was most likely the main pathway for methane production in RCH<sub>4</sub>. The high level of CHT (2.54 g L<sup>-1</sup>) in fermented molasses associated with the high abundance of fermenters (28.3% of overall community) and genes encoding for Fe-hydrogenase present over time (Table 2) strongly suggests that hydrogen was produced mainly by fermentation of residual CHT of fermented molasses. Accumulation of HLa, HAc and HPt at all operating phases and the low abundance of syntrophs (7.5% of overall community) shows that at OLR higher than 6.9 kg COD m<sup>-3</sup> d<sup>-1</sup> syntrophic activity was inefficient. Syntrophs were not able to handle the acids produced by fermentation of CHT associated with acids from the fermented molasses. The inefficient syntrophic activity impaired methane production with decreased numbers of McrA genes (Table 2) and MY values.

To analyze the correlation of microbiota with monitoring parameters, CCA was also carried out in the methanogenic reactor (Fig. 8). Most genera have benefited from the increase in pH, both by pH adjustment and the consumption of acids, including *Methanothermobacter*. Most isolated members of this genus develop optimally at pH higher than 7.0, and either this factor or the consumption of organic acids influences these archaea strongly. Several groups including *Ureibacillus*,



**Fig. 8.** Canonical correspondence analysis (CCA) carried out with monitoring and microbial data obtained from RCH4.

*Coprothermobacter*, *Fervidobacterium*, *Bacteroides* and *Dysgonomas* correlated positively with acids and the VMPR. This could imply that key microorganisms degrading organic matter (particularly syntrophs) are more important to methane production than the methanogenic archaea directly, as previously discussed.

The functional inference over the taxonomic data from the methanogenic reactor reiterates how little it is known about the groups composing this community. Complementary metabolic function data are presented in the Supplementary data section. Most of the recognizable functions include fermentation as a possible metabolism, suggesting that the further degradation of organic matter is an important driver of this community, particularly considering the residual CH<sub>4</sub> concentrations observed. The abundance of methanogenesis correlates closely to the *Methanothermobacter* abundance, corroborating their role as the main methanogen. Thiosulfate respiration, sulfate respiration and nitrate reduction plays a minor role as alternative metabolism to several microbes in the reactor in lower abundances, as consequence of residual CH<sub>4</sub> concentrations. Aerobic chemoheterotrophy capacity is also present within the microbial population, and likely contributes to maintain the anaerobic environment by consuming any available oxygen.

### 3.3. Mass and energy balances

$MB_{COD}$  values calculated for both reactors are depicted in Fig. 9a. A global analysis is presented for  $RH_2$  due to the operation under a fixed OLR, whilst in  $RCH_4$  the  $MB_{COD}$  was calculated according to the different operating phases (except for phase I). Residual CH<sub>t</sub> and metabolite concentrations corresponded to the highest detected fractions in  $RH_2$ , i.e., 27% and 18%, respectively, whilst the contribution of bioH<sub>2</sub> was approximately 1% (Fig. 9a). Previous studies on vinasse-fed thermophilic acidogenic systems indicated similar proportions of bioH<sub>2</sub> in the

Table 2

**Table 2**  
Gene copy numbers for Fe-hydrogenase and McrA in DNA samples taken from the RCH<sub>4</sub> during the operating period.

Biomass sample	Inoculum	OLR (kg COD m <sup>-3</sup> d <sup>-1</sup> )						
		1.0	2.3	4.6	6.9	15.8	25.2	18.5
Log n. copies of genes encoding for Fe-hydrogenase ng <sup>-1</sup> DNA	—	—	9.0	8.7	9.0	8.5	8.8	8.2
Log n. copies of McrA genes ng <sup>-1</sup> DNA	8.6	9.4	10.0	9.0	8.5	8.2	*	6.3

Note: \* <26,600 n. copies.

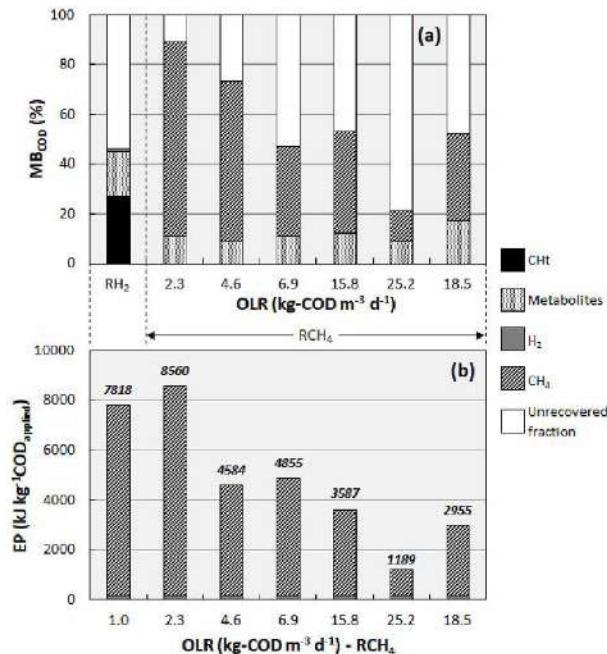


Fig. 9. (a) COD-base mass balance (MB<sub>COD</sub>) and (b) energetic potential (EP) associated with bioH<sub>2</sub> and methane production in all operating conditions (numbers in italic style indicate the global energetic potential – EP<sub>global</sub>).

MB<sub>COD</sub> (0.5% [38]; 0.6% [39]), and this low contribution could be associated with numerous factors, such as the bioH<sub>2</sub> retention within the support material and its metabolic consumption (e.g. homoacetogenesis) [38]. The relatively high unrecovered fraction (54%; Fig. 9a) was particularly relevant in the effluent COD, as CHt and metabolite levels corresponded to approximately 50% of the COD in fermented molasses. Given the high coverage of the analytical methods used, i.e., the capability to detect a wide range of organic compounds of AD systems, this pattern could result from the contribution of melanoidins. Melanoidins are macromolecular products from the Maillard reaction between sugars and proteins at high temperatures, being characterized by a high recalcitrance to anaerobic populations [69]. The occurrence of melanoidins was previously associated with the undetected COD fraction of mass balances calculated for reactors fed with sugarcane-derived byproducts, namely, molasses [19] and vinasse [16,38].

The fraction of soluble metabolites remained approximately constant (12%) throughout the entire operating period in RCH<sub>4</sub>, whilst distinct patterns were observed for the contribution of methane and unrecovered fractions to the MB<sub>COD</sub> (Fig. 9a). Methane production accounted for the largest proportions (64–78%) of the MB<sub>COD</sub> up to the OLR of 4.6 kg COD m<sup>-3</sup> d<sup>-1</sup> (Fig. 9a). The application of higher OLR markedly decreased methane fractions (<41%; Fig. 9a), which was concomitantly followed by an increase in unrecovered proportions of the metabolized substrate (up to 79% during the application of the highest COD (25.2 kg COD m<sup>-3</sup> d<sup>-1</sup>; Fig. 9a). The varying, but neglectable contribution of soluble phase metabolites coupled to both the increase in the unrecovered fraction and the negligible VSS losses in the system, confirm the previous hypothesis regarding the retention of biomass within the AnSTBR. This hypothesis is due to the great coverage of the characterization methods employed in this study, mainly the determination of VFA by HPLC, which included, in addition to HFo, HAc, HPr, HLa, and HBu, the detection of citric, malic, succinic, iso-butyric, valeric, iso-valeric and caproic acids, as well as methanol, ethanol and n-butanol.

The energetic assessment indicated an average EP<sub>H2</sub> of 153 kJ kg<sup>-1</sup>COD<sub>applied</sub> whilst EP<sub>CH4</sub> values varied within the range 1036–8407

kJ kg<sup>-1</sup>COD<sub>applied</sub> depending on the applied OLR in RCH<sub>4</sub> (Fig. 9b). BioH<sub>2</sub> contribution to the global energy potential increased (1.8–14.8%) as the performance of the methanogenic populations was impaired, with most of values below 5%, which is consistent with the literature on two-stage AD [13,14,26,70]. The energy recovery potential of AD applied to sugarcane byproducts, namely, molasses and vinasse, is compared in Table 3. EP<sub>H2</sub> values indicate a relatively low energetic efficiency of RH<sub>2</sub>, regarding both types of substrates. Despite the long-term bioH<sub>2</sub> production levels, substrate conversion efficiency in RH<sub>2</sub> was low (<50%), as detailed in Section 3.1, requiring further improvements to better exploit molasses as a substrate for bioH<sub>2</sub>-producing populations.

The highest EP<sub>global</sub> (8560 kJ kg<sup>-1</sup>COD<sub>applied</sub>) was observed in the application of an OLR of 2.3 kg COD m<sup>-3</sup> d<sup>-1</sup> in RCH<sub>4</sub> (Fig. 9b), reaching a higher value than the ones estimated for vinasse (Table 3). Overall, the results indicate that applying low OLR (2.2–5.7 kg COD m<sup>-3</sup> d<sup>-1</sup>) in molasses-fed reactors promptly provides EP<sub>CH4</sub> values (8560–9899 kJ kg<sup>-1</sup>COD<sub>applied</sub>) higher than those (4797–6902 kJ kg<sup>-1</sup>COD<sub>applied</sub>) achieved at much higher OLR in vinasse-fed AD systems (>20.0 kg COD m<sup>-3</sup> d<sup>-1</sup>) (Table 3). This characterizes molasses as a highly-efficient substrate for AD systems. The observed discrepancy results from compositional specificities in both substrates, i.e., the higher refractory organic fraction found in vinasse forces the application of higher OLR to provide enough amounts of biodegradable organic matter to anaerobic populations. Conversely, the availability of easily degradable organic matter is promptly high at low OLR in molasses-fed systems, which provides high energy recovery potential levels in these conditions. Nevertheless, higher substrate (carbohydrate) conversion efficiencies are still required in acidogenesis, aiming to both increase the energetic efficiency and use more compact units when applying higher OLR in methanogenic systems.

#### 4. Conclusions and future prospects

Long-term bioH<sub>2</sub> and methane production was successfully achieved from molasses thermophilic two-stage biodigestion. The results show that combining the application of high OLR (80–120 kg COD m<sup>-3</sup> d<sup>-1</sup>), low pH (4.0–5.0) and high temperature directly favor the interaction between bioH<sub>2</sub> (*Clostridial* populations) and lactate-producing bacteria in acidogenic systems fed with sugarcane molasses. The co-fermentation of lactate and acetate plays a relevant role in bioH<sub>2</sub> production. The full optimization of the operating conditions, namely, OLR, HRT and pH, may be a key approach to combine efficient substrate conversion and maximum bioH<sub>2</sub> production. Following the course of substrate conversion, biomass acclimatization was associated with carbon sequestration in the methanogenic phase, in order to maintain high COD removal levels (70–80%) even at impaired methane production conditions. Using an inoculum acclimatized to thermophilic temperature will provide more efficient methanogenic activities at higher OLR in the methanogenic stage, specifically by stimulating populations of syntrophs and acetoclastic methanogens. Studies on the biomass retention evolution are also required to better understand aspects of biomass growth in methanogenic reactors, potentially providing basis to correlate the occurrence of organic overloading conditions with both the effective substrate availability and the microbial communities established. Regarding energetic aspects, the results indicate the potential to achieve higher energy generation levels from molasses compared to vinasse at much lower OLR (<5.0 vs. >20.0 kg COD m<sup>-3</sup> d<sup>-1</sup>), which is favored by a high biodegradable organic content. In this case, future scenario-based studies on the potential use of molasses-derived biogas are required to direct full-scale applications, providing data to support both techno-economical assessments and comparative analyses with conventional ethanol-producing steps. Such aspects are imperative to encourage the application of diversified approaches in the processing of sugarcane-derived substrates, focusing on the use of anaerobic digestion as the core step.

**Table 3**

Comparative analysis of the energetic potential associated with the application of AD in the processing of sugarcane byproducts.

Byproduct	Acidogenic stage		Methanogenic stage		EP <sub>global</sub> <sup>b</sup>	Reference
	OLR <sup>a</sup> (Temperature)	EP <sub>H2</sub> <sup>b</sup>	OLR <sup>c</sup> (Temperature)	EP <sub>CH4</sub> <sup>b</sup>		
Molasses	112.0 (35 °C)	268 <sup>c</sup>	5.7 (35 °C)	9899 <sup>c</sup>	10,167 <sup>c</sup>	Park et al. [71]
	240.0 (30 °C)	203 <sup>c</sup>	–	–	–	Freitas et al. [17]
	60.0 (55 °C)	381 <sup>c</sup>	–	–	–	Oliveira et al. [18]
	120.0 (55 °C)	153	2.2 (55 °C)	8407	8560	This study
Vinasse	72.4 (55 °C)	78 <sup>c</sup>	–	–	–	Ferraz Jr. et al. [22]
	84.2 (55 °C)	98 <sup>c</sup>	25.0 (55 °C)	4696 <sup>c</sup>	4794 <sup>c</sup>	Ferraz Jr. et al. [13,39]
	84.2 (55 °C)	153 <sup>c</sup>	25.0 (55 °C)	6637 <sup>c</sup>	6771 <sup>c</sup>	Fuess et al. [14,38]
	87.5 (55 °C)	254 <sup>c</sup>	–	–	–	Fuess et al. [16]
	60.0 (55 °C)	82 <sup>d</sup>	21.6 (55 °C)	6820 <sup>d</sup>	6902 <sup>d</sup>	Ramos and Silva [70]

Abbreviations: OLR: organic loading rate, EP<sub>H2</sub>: bioH<sub>2</sub> energy potential, EP<sub>CH4</sub>: CH<sub>4</sub> energy potential, EP<sub>global</sub>: global energy potential.

Notes.

<sup>a</sup> kg COD m<sup>-3</sup> d<sup>-1</sup>.<sup>b</sup> kJ kg<sup>-1</sup>COD<sub>applied</sub>.<sup>c</sup> Calculated from available data in the reference.<sup>d</sup> Data presented by authors.**Credit author statement**

**Rogério S. Vilela:** Investigation (reactor monitoring). **Lucas T. Fuess:** Conceptualization, Methodology (energy potential assessment), writing (original draft preparation, review & editing), Visualization. **Flavia T. Saia:** Methodology (DNA extraction), Visualization (metagenomics and statistics), writing (review), Funding acquisition. **Catarina R.M. Silveira:** Investigation (bioinformatics), Software (bioinformatics), Visualization (metagenomics). **Cristiane A. Oliveira:** Conceptualization, Methodology (mass balance), writing (original draft). **Pedro A. Andrade:** Methodology (qPCR). **Alette Langenhoff:** writing (review), Funding acquisition. **Bas van der Zaan:** Methodology (qPCR), writing (review). **Fabio Cop:** Formal analysis (statistics), Visualization (statistics). **Gustavo B. Gregoracci:** Methodology (bioinformatics), Software (bioinformatics), Resources (computing), Visualization (metagenomics and statistics), writing (review). **Marcia H.R.Z. Damianovic:** Supervision, writing (review), Funding acquisition.

**Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Acknowledgments**

This work was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, Brazil) [grant number 2012/51496-3] in collaboration with BE-Basic (The Netherlands) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil).

**Appendix A. Supplementary data**

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.rser.2021.110974>.

**References**

- [1] Hussain A, Arif SM, Aslam M. Emerging renewable and sustainable energy technologies: state of the art. *Renew Sustain Energy Rev* 2017;71:12–28. <https://doi.org/10.1016/j.rser.2016.12.033>.
- [2] Panwar NL, Kaushik SC, Kothari S. Role of renewable energy sources in environmental protection: a review. *Renew Sustain Energy Rev* 2011;15(3):1513–24. <https://doi.org/10.1016/j.rser.2010.11.037>.
- [3] Dias MOS, Maciel Filho R, Mantelatto PE, Cavalett O, Rossell CEV, Bonomi A, et al. Sugarcane processing for ethanol and sugar in Brazil. *Environ Dev* 2015;15:35–51. <https://doi.org/10.1016/j.envdev.2015.03.004>.
- [4] Fuess LT, Garcia ML. Implications of stillage land disposal: a critical review on the impacts of fertigation. *J Environ Manag* 2014;145:210–29. <https://doi.org/10.1016/j.jenvman.2014.07.003>.
- [5] Del Nery V, Alves I, Damjanovic MHRZ, Pires EC. Hydraulic and organic rates applied to pilot scale UASB reactor for sugar cane vinasse degradation and biogas generation. *Biomass Bioenergy* 2018;119:411–7. <https://doi.org/10.1016/j.biombioe.2018.10.002>.
- [6] Fuess LT, Zaiat M. Economics of anaerobic digestion for processing sugarcane vinasse: applying sensitivity analysis to increase process profitability in diversified biogas applications. *Process Saf Environ Protect* 2018;115:27–37. <https://doi.org/10.1016/j.psep.2017.08.007>.
- [7] Moraes BS, Zaiat M, Bonomi A. Anaerobic digestion of vinasse from sugarcane ethanol production in Brazil: challenges and perspectives. *Renew Sustain Energy Rev* 2015;44:888–903. <https://doi.org/10.1016/j.rser.2015.01.023>.
- [8] Klein BC, Chagas MF, Watanabe MDB, Bonomi A, Maciel Filho R. Low carbon biofuels and the New Brazilian National Biofuel Policy (RenovaBio): a case study for sugarcane mills and integrated sugarcane-microalgae biorefineries. *Renew Sustain Energy Rev* 2019;115:109365. <https://doi.org/10.1016/j.rser.2019.109365>.
- [9] Fuess LT, Klein BC, Chagas MF, Rezende MCAF, Garcia ML, Bonomi A, et al. Diversifying the technological strategies for recovering bioenergy from the two-phase anaerobic digestion of sugarcane vinasse: an integrated techno-economic and environmental approach. *Renew Energy* 2018;122:674–87. <https://doi.org/10.1016/j.renene.2018.02.003>.
- [10] Moraes BS, Junqueira TL, Pavanello LG, Cavalett O, Mantelatto PE, Bonomi A, et al. Anaerobic digestion of vinasse from sugarcane biorefineries in Brazil from energy, environmental, and economic perspectives: profit or expense? *Appl Energy* 2014;113:825–35. <https://doi.org/10.1016/j.apenergy.2013.07.018>.
- [11] ANP. Resolução ANP n. 8, de 30 de janeiro de 2015 – regulamenta o uso de biometano no Brasil [Resolution ANP n. 8, January 30th 2015 – regulates the use of biomethane in Brazil]. Brasília, Brazil: Diário Oficial da União 2015;22:100–1 [in Portuguese], <http://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?data=02/02/2015&jornal=1&pagina=100&totalArquivos=156>. [Accessed 29 March 2020].
- [12] Junqueira TL, Moraes B, Gouveia VLR, Chagas MF, Morais ER, Watanabe MDB, et al. Use of VSB to plan research programs and public policies. In: Bonomi A, Cavalett O, Cunha MP, Lima MAP, editors. *Virtual biorefinery: an optimization strategy for renewable carbon valorization*. first ed. New York: Springer; 2016. p. 257–82.
- [13] Ferraz Jr ADN, Koyama MH, Araújo Jr MM, Zaiat M. Thermophilic anaerobic digestion of raw sugarcane vinasse. *Renew Energy* 2016;89:245–52. <https://doi.org/10.1016/j.renene.2015.11.064>.
- [14] Fuess LT, Kiyanu LSM, Ferraz Jr ADN, Persinoti GF, Squina FM, Garcia ML, et al. Thermophilic two-phase anaerobic digestion using an innovative fixed-bed reactor for enhanced organic matter removal and bioenergy recovery from sugarcane vinasse. *Appl Energy* 2017;189:480–91. <https://doi.org/10.1016/j.apenergy.2016.12.071>.
- [15] Yu B, Shan A, Zhang D, Lou Z, Yuan H, Huang X, et al. Dosing time of ferric chloride to disinhibit the excessive volatile fatty acids in sludge thermophilic anaerobic digestion system. *Bioresour Technol* 2015;189:154–61. <https://doi.org/10.1016/j.biortech.2015.03.144>.
- [16] Fuess LT, Zaiat M, Nascimento CAO. Novel insights on the versatility of biohydrogen production from sugarcane vinasse via thermophilic dark fermentation: impacts of pH-driven operating strategies on acidogenesis metabolite profiles. *Bioreesour Technol* 2019;286:121379. <https://doi.org/10.1016/j.biortech.2019.121379>.
- [17] Freitas IBF, Menezes CA, Silva EL. An alternative for value aggregation to the sugarcane chain: biohydrogen and volatile fatty acids production from sugarcane molasses in mesophilic expanded granular sludge bed reactors. *Fuel* 2020;260:116419. <https://doi.org/10.1016/j.fuel.2019.116419>.

- [18] Oliveira CA, Fuess LT, Soares LA, Damianovic MHRZ. Thermophilic biohydrogen production from sugarcane molasses under low pH: metabolic and microbial aspects. *Int J Hydrogen Energy* 2020;45(7):4182–92. <https://doi.org/10.1016/j.ijhydene.2019.12.013>.
- [19] Vilela R, Saia FT, Gregoracci GB, Duarte R, Andrade P, van der Zaan B, et al. Hydrogen production in reactors: the influence of organic loading rate, inoculum and support material. *Int J Hydrogen Energy* 2019;44(50):27259–71. <https://doi.org/10.1016/j.ijhydene.2019.08.180>.
- [20] Wilkie AC, Riedesel KJ, Owens JM. Stillage characterization and anaerobic treatment of ethanol stillage from conventional and cellulosic feedstocks. *Biomass Bioenergy* 2000;19(2):63–102. [https://doi.org/10.1016/S0961-9534\(00\)0017-9](https://doi.org/10.1016/S0961-9534(00)0017-9).
- [21] Amani T, Nosrati M, Mousavi SM, Elyasi Sh. Study of microbiological and operational parameters in thermophilic syntrophic degradation of volatile fatty acids in an upflow anaerobic sludge blanket reactor. *J Environ Chem Eng* 2015;3(1):507–14. <https://doi.org/10.1016/j.jece.2014.12.016>.
- [22] Ferraz Jr ADN, Wenzel J, Etchebehere C, Zaiat M. Effect of organic loading rate on hydrogen production from sugarcane vinasse in thermophilic acidogenic packed bed reactors. *Int J Hydrogen Energy* 2014;39(30):16852–62. <https://doi.org/10.1016/j.ijhydene.2014.08.017>.
- [23] Pawar SS, van Niel EWJ. Thermophilic biohydrogen production: how far are we? *Appl Microbiol Biotechnol* 2013;97(18):7999–8009. <https://doi.org/10.1007/s00253-013-5141-1>.
- [24] Aquino S, Fuess LT, Pires EC. Media arrangement impacts cell growth in anaerobic fixed-bed reactors treating sugarcane vinasse: structured vs. random biomass immobilization. *Bioresour Technol* 2017;235:219–28. <https://doi.org/10.1016/j.biortech.2017.03.120>.
- [25] Fuess LT, Ferraz Jr ADN, Machado CB, Zaiat M. Temporal dynamics and metabolic correlation between lactate-producing and hydrogen-producing bacteria in sugarcane vinasse dark fermentation: the key role of lactate. *Bioresour Technol* 2018;247:426–33. <https://doi.org/10.1016/j.biortech.2017.09.121>.
- [26] Kongjan P, O-Thong S, Angelidaki I. Hydrogen and methane production from desugared molasses using a two-stage thermophilic anaerobic process. *Eng Life Sci* 2013;13(2):118–25. <https://doi.org/10.1002/elsc.201100191>.
- [27] APHA, AWWA, WEF. Standard methods for the examination of water and wastewater. twenty-second ed. Washington, DC: APHA; 2012.
- [28] Ripley LE, Boyle WC, Converse JC. Improved alkalimetric monitoring for anaerobic digestion of high-strength wastes. *J Water Pollut Control Fed* 1986;58(5):406–11.
- [29] Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric methods for determination of sugar and related substance. *Anal Chem* 1956;28(3):350–6. <https://doi.org/10.1021/ac60111a017>.
- [30] Penteado ED, Lazaro CZ, Sakamoto IK, Zaiat M. Influence of seed sludge and pretreatment method on hydrogen production in packed-bed anaerobic reactors. *Int J Hydrogen Energy* 2013;38(14):6137–45. <https://doi.org/10.1016/j.ijhydene.2013.01.067>.
- [31] Perna V, Castelló E, Wenzel J, Zampol C, Fontes Lima DM, Borzacconi L, et al. Hydrogen production in an upflow anaerobic packed bed reactor used to treat cheese whey. *Int J Hydrogen Energy* 2013;38(1):54–62. <https://doi.org/10.1016/j.ijhydene.2012.10.022>.
- [32] Fuess LT, Garcia ML, Zaiat M. Seasonal characterization of sugarcane vinasse: Assessing environmental impacts from fertirrigation and the bioenergy recovery potential through biodigestion. *Sci Total Environ* 2018;634:29–40. <https://doi.org/10.1016/j.scitotenv.2018.03.326>.
- [33] Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, et al. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res* 2012;41(1):e1. <https://doi.org/10.1093/nar/gks808>.
- [34] Louca S, Parfey LW, Doebele M. Decoupling function and taxonomy in the global ocean microbiome. *Science* 2016;353(6305):1272–7. <https://doi.org/10.1126/science.aaf4507>.
- [35] Legendre P, Legendre L. Numerical ecology. third ed., ume 24. Amsterdam, Oxford: Elsevier; 2012.
- [36] Fang HH, Zhang T, Li C. Characterization of Fe-hydrogenase genes diversity and hydrogen-producing population in an acidophilic sludge. *J Biotechnol* 2006;126(3):357–64. <https://doi.org/10.1016/j.jbiotec.2006.04.023>.
- [37] Hales BA, Edwards C, Ritchie DA, Hall G, Pickup RW, Saunders JR. Isolation and identification of methanogen-specific DNA from blanket bog peat by PCR amplification and sequence analysis. *Appl Environ Microbiol* 1996;62(2):668–75.
- [38] Fuess LT, Kiyuna LSM, Garcia ML, Zaiat M. Operational strategies for long-term biohydrogen production from sugarcane stillage in continuous acidogenic packed-bed reactor. *Int J Hydrogen Energy* 2016;41(19):8132–45. <https://doi.org/10.1016/j.ijhydene.2015.10.143>.
- [39] Ferraz Jr ADN, Etchebehere C, Zaiat M. High organic loading rate on thermophilic hydrogen production and metagenomic study at an anaerobic packed-bed reactor treating a residual liquid stream of a Brazilian biorefinery. *Bioresour Technol* 2015;186:81–8. <https://doi.org/10.1016/j.biortech.2015.03.035>.
- [40] Saady NMC. Homoacetogenesis during hydrogen production by mixed cultures dark fermentation. *Int J Hydrogen Energy* 2013;38(30):13172–91. <https://doi.org/10.1016/j.ijhydene.2013.07.122>.
- [41] Matsumoto M, Nishimura Y. Hydrogen production by fermentation using acetic acid and lactic acid. *J Biosci Bioeng* 2007;103(3):236–41. <https://doi.org/10.1263/jbb.103.236>.
- [42] Braga AFM, Ferraz Jr ADN, Zaiat M. Thermophilic biohydrogen production using a UASB reactor: performance during long-term operation. *J Chem Technol Biotechnol* 2016;91(4):967–76. <https://doi.org/10.1002/jctb.4665>.
- [43] Chojnacka A, Blaszczyk MK, Szczesny P, Nowak K, Sumitrska M, Tomczyk-Żak K, et al. Comparative analysis of hydrogen-producing bacterial biofilms and granular sludge formed in continuous cultures of fermentative bacteria. *Bioresour Technol* 2011;102(21):10057–64. <https://doi.org/10.1016/j.biortech.2011.08.063>.
- [44] Yang G, Wang J. Changes in microbial community structure during dark fermentative hydrogen production. *Int J Hydrogen Energy* 2019;44(47):25542–50. <https://doi.org/10.1016/j.ijhydene.2019.08.039>.
- [45] O-Thong S, Prasertsan P, Karakashev D, Angelidaki I. Thermophilic fermentative hydrogen production by the newly isolated *Thermoanaerobacterium thermosaccharolyticum* PSU-2. *Int J Hydrogen Energy* 2008;33(4):1204–14. <https://doi.org/10.1016/j.ijhydene.2007.12.015>.
- [46] Castelló E, Ferraz-Junior ADN, Andreani C, Anzola-Rojas MP, Borzacconi L, Buitrón G, et al. Stability problems in the hydrogen production by dark fermentation: possible causes and solutions. *Renew Sustain Energy Rev* 2020;119:109602. <https://doi.org/10.1016/j.rser.2019.109602>.
- [47] Fang HH, Liu H, Zhang T. Characterization of a hydrogen-producing granular sludge. *Biotechnol Bioeng* 2002;78(1):44–52. <https://doi.org/10.1002/bit.10174>.
- [48] Angelidaki I, Sanders W. Assessment of the anaerobic biodegradability of macropolutants. *Rev Environ Sci Biotechnol* 2004;3:117–29. <https://doi.org/10.1007/s11157-004-2502-3>.
- [49] Dell MMR, Foresti E. Efeito do bicarbonato de sódio no tratamento de vinhaça em AnSBRR operado a 55 e 35°C [Effect of the sodium bicarbonate in the treatment of vinasse in AnSBRR operated at 55 and 35°C]. *Eng Sanitária Ambient* 2010;15(3):275–82. <https://doi.org/10.1590/S1413-41522010000300011>.
- [50] van Lier JB, Hulshof J, Stams AJM, Lettinga G. Temperature susceptibility of thermophilic methanogenic sludge: implications for reactor start-up and operation. *Bioresour Technol* 1993;43(3):227–35. [https://doi.org/10.1016/0960-8524\(93\)90035-A](https://doi.org/10.1016/0960-8524(93)90035-A).
- [51] Chojnacka A, Szczęsny P, Blaszczyk MK, Zielenkiewicz U, Detman A, Salamon A, et al. Noteworthy facts about a methane-producing microbial community processing acidic effluent from sugar beet molasses fermentation. *PLoS One* 2015;10(5):e0128008. <https://doi.org/10.1371/journal.pone.0128008>.
- [52] Mau I, Koek DE, Cibis KG, Hahnke S, Kim YS, Langer T, et al. Unraveling the microbiome of a thermophilic biogas plant by metagenome and metatranscriptome analysis complemented by characterization of bacterial and archaeal isolates. *Biotechnol Biofuels* 2016;9:171. <https://doi.org/10.1186/s13068-016-0581-3>.
- [53] Barros VG, Duda RM, Vantini JS, Omori WP, Ferro MIT, Oliveira RA. Improved methane production from sugarcane vinasse with filter cake in thermophilic UASB reactors, with predominance of *Methanothermobacter* and *Methanosarcina* archaea and *Thermotoga* bacteria. *Bioresour Technol* 2017;244(1):371–81. <https://doi.org/10.1016/j.biortech.2017.07.106>.
- [54] Bovio P, Cabecas A, Etchebehere C. Preliminary analysis of *Chloroflexi* populations in full-scale UASB methanogenic reactors. *J Appl Microbiol* 2019;126(2):667–83. <https://doi.org/10.1111/jam.14115>.
- [55] Oosterkamp MJ, Méndez-García C, Kim CH, Bauer S, Ibáñez AB, Zimmerman S, et al. Lignocellulose-derived thin stillage composition and efficient biological treatment with a high-rate hybrid anaerobic bioreactor system. *Biotechnol Biofuels* 2016;9:120. <https://doi.org/10.1186/s13068-016-0532-z>.
- [56] Oosterkamp MJ, Bauer S, Ibáñez AB, Méndez-García C, Hong PY, Cann I, et al. Identification of methanogenesis and syntrophy as important microbial metabolic processes for optimal thermophilic anaerobic digestion of energy cane thin stillage. *Bioresour Technol* 2019;7:100254. <https://doi.org/10.1016/j.biortech.2019.100254>.
- [57] Watthanalamloet A, Tachaapaikoon C, Lee SY, Kosugi A, Mori Y, Tanasupawat S, et al. *Cellulosibacter alkalithermophilic* gen. nov., sp. nov., an anaerobic alkalithermophilic, cellulolytic-xylanolytic bacterium isolated from soil of a coconut garden. *Int J Syst Evol Microbiol* 2012;62(2):2330–5. <https://doi.org/10.1093/ijs/0.027854.0>.
- [58] Grégoire P, Fardeau ML, Joseph M, Guasco S, Hamade F, Biasutti S, et al. Isolation and characterization of *Thermaracetothrix daxensis* gen. nov., sp. nov., a thermophilic anaerobic bacterium pertaining to the phylum "Chloroflexi", isolated from a deep hot aquifer in the Aquitaine Basin. *Syst Appl Microbiol* 2011;34(7):494–7. <https://doi.org/10.1016/j.syapm.2011.02.004>.
- [59] Karlsson FH, Ussery DW, Nielsen J, Nookaei I. A closer look at *Bacteroides*: phylogenetic relationships and genomic implications of a life in the human gut. *Microb Ecol* 2011;61:473–85. <https://doi.org/10.1007/s00248-010-9796-1>.
- [60] Moset V, Poulsen M, Wahid R, Højberg O, Möller HB. Mesophilic versus thermophilic anaerobic digestion of cattle manure: methane productivity and microbial ecology. *Microb Biotechnol*. 2015;8(5):787–800. <https://doi.org/10.1111/1751-7915.12271>.
- [61] Frock AD, Notey JS, Kelly RM. The genus *Thermotoga*: recent developments. *Environ Technol* 2010;31(10):1169–81. <https://doi.org/10.1080/09593330.2010.484076>.
- [62] Gagliano MC, Braguglia CM, Petruccioli M, Rossetti S. Ecology and biotechnological potential of the thermophilic fermentative *Copriothermobacter* spp. *FEMS Microbiol Ecol* 2015;91(5):fiv018. <https://doi.org/10.1093/fems/fiv018>.
- [63] Hattori S, Kamagata Y, Hanada S, Shoun H. *Thermacetogenium phaeum* gen. nov., sp. nov., a strictly anaerobic, thermophilic, syntrophic acetate-oxidizing bacterium. *Int J Syst Evol Microbiol* 2000;50(4):1601–9. <https://doi.org/10.1099/00207713-50-4-1601>.
- [64] Sekiguchi Y, Kamagata Y, Nakamura K, Ohashi A, Harada H. *Syntrophothermus lipocalidus* gen. nov., sp. nov., a novel thermophilic, syntrophic, fatty-acid-oxidizing anaerobe which utilizes isobutyrate. *Int J Syst Evol Microbiol* 2000;50(2):771–9. <https://doi.org/10.1099/00207713-50-2-771>.
- [65] Sekiguchi Y, Imachi H, Susilorkumi A, Muramatsu M, Ohashi A, Harada H, et al. *Tepidanaerobacter syntrophicus* gen. nov., sp. nov., an anaerobic, moderately thermophilic, syntrophic alcohol- and lactate-degrading bacterium isolated from thermophilic digested sludges. *Int J Syst Evol Microbiol* 2006;56(7):1621–9. <https://doi.org/10.1099/ijss.0.64112-0>.

- [66] Westerholm M, Roos S, Schnürer A. *Syntrophaceticus schinkii* gen. nov., sp. nov., an anaerobic, syntrophic acetate-oxidizing bacterium isolated from a mesophilic anaerobic filter. FEMS Microbiol Lett 2010;309(1):100–4. <https://doi.org/10.1111/j.1574-6968.2010.02023.x>.
- [67] Schmidt A, Müller N, Schink B, Schleheck D. A proteomic view at the biochemistry of syntrophic butyrate oxidation in *Syntrophomonas wolfei*. PloS One 2013;8(2):e56905. <https://doi.org/10.1371/journal.pone.0056905>.
- [68] Detman A, Mielecki D, Pleśniak E, Bucha M, Janiga M, Matyasik I, et al. Methane-yielding microbial communities processing lactate-rich substrates: a piece of the anaerobic digestion puzzle. Biotechnol Biofuels 2018;11:116. <https://doi.org/10.1186/s13068-018-1106-z>.
- [69] Peña M, Coca M, González G, Rioja R, García MT. Chemical oxidation of wastewater from molasses fermentation with ozone. Chemosphere 2003;51(9):893–900. [https://doi.org/10.1016/S0045-6535\(03\)00159-0](https://doi.org/10.1016/S0045-6535(03)00159-0).
- [70] Ramos LR, Silva EL. Thermophilic hydrogen and methane production from sugarcane stillage in two-stage anaerobic fluidized bed reactors. Int J Hydrogen Energy 2020;45(8):5239–51. <https://doi.org/10.1016/j.ijhydene.2019.05.025>.
- [71] Park MJ, Jo JH, Park D, Lee DS, Park JM. Comprehensive study on a two-stage anaerobic digestion process for the sequential production of hydrogen and methane from cost-effective molasses. Int J Hydrogen Energy 2010;35(12):6194–202. <https://doi.org/10.1016/j.ijhydene.2010.03.135>.



**13<sup>th</sup>**

**World Congress  
on Anaerobic Digestion**

**Recovering (bio) Resources  
for the World.**



**International  
Water Association**

## **Certificate**

**MR./MS. ROGERIO VILELA**

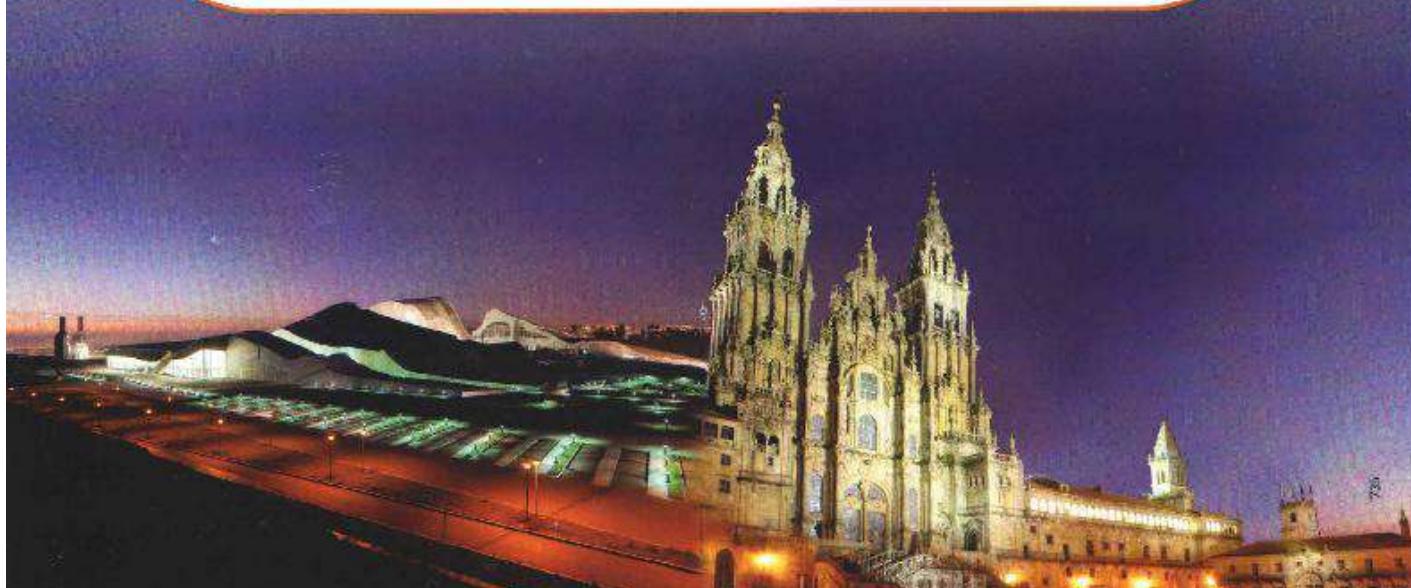
University of São Paulo

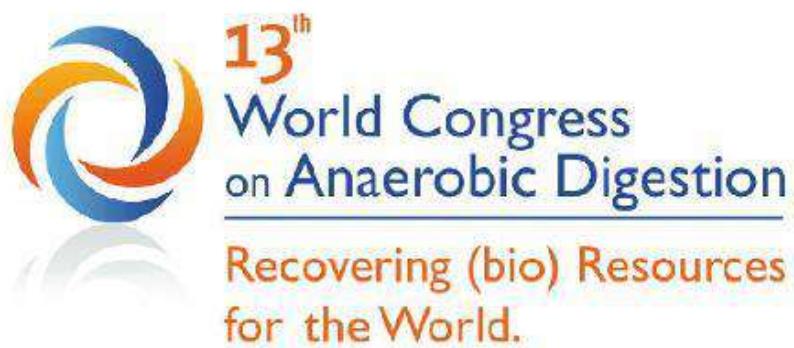
has successfully participated in the **13th World Congress on Anaerobic  
Digestion, Recovering (bio) Resources for the World,**  
held in Santiago de Compostela on June 25th-28th, 2013.

Santiago de Compostela, June 28th, 2013

Juan M. Lema

Chair of the Organising Committee





# Certificate

**Mr. ROGERIO SILVEIRA VILELA**

University of São Paulo

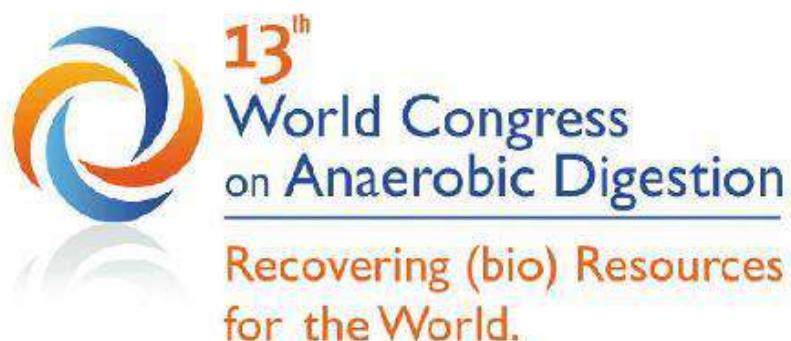
has presented the paper "Biological organic matter removal and sulfate reduction from sulfate-rich wastewater in horizontal-flow anaerobic immobilized biomass reactor (HAIB)" (R. S. Vilela, M. H. R. Z. Damianovic, E. Foresti) as Poster Presentation at the IWA international Conference 13<sup>th</sup> World Congress on Anaerobic Digestion, Recovering (bio) Resources for the World, held in Santiago de Compostela on 25<sup>th</sup>-28<sup>th</sup> June, 2013.

Santiago de Compostela, 28<sup>th</sup> June, 2013

Juan M. Lema

Chair of the Organising Committee





# Certificate

**Mr. ROGERIO SILVEIRA VILELA**

University of São Paulo

has presented the paper "Organic matter removal from wastewater with high sulfate concentration by sulphidogenic and methanogenic combined pathways" (R. S. Vilela, M. H. R. Z. Damianovic, E. Foresti) as *Platform Presentation* at the IWA international Conference 13<sup>th</sup> World Congress on Anaerobic Digestion, Recovering (bio) Resources for the World, held in Santiago de Compostela on 25<sup>th</sup>-28<sup>th</sup> June, 2013.

Santiago de Compostela, 28<sup>th</sup> June, 2013

Juan M. Lema

Chair of the Organising Committee





**CERTIFICATE OF ATTENDANCE**

This is to certify that

**Rogerio Vilela**

Attended the 14<sup>th</sup> World Congress on Anaerobic Digestion held in Viña del Mar,  
Chile,  
from November 15<sup>th</sup> to 18<sup>th</sup>, 2015.

Dr. Rolando Chamy  
Chairman  
14th World Congress on Anaerobic Digestion

**Closing cycles for sustainability**



Closing cycles for sustainability



World Congress  
on Anaerobic  
Digestion

Presented to

**R. Vilela, F. T. Saia and M. H. R. Z. Damianovic**

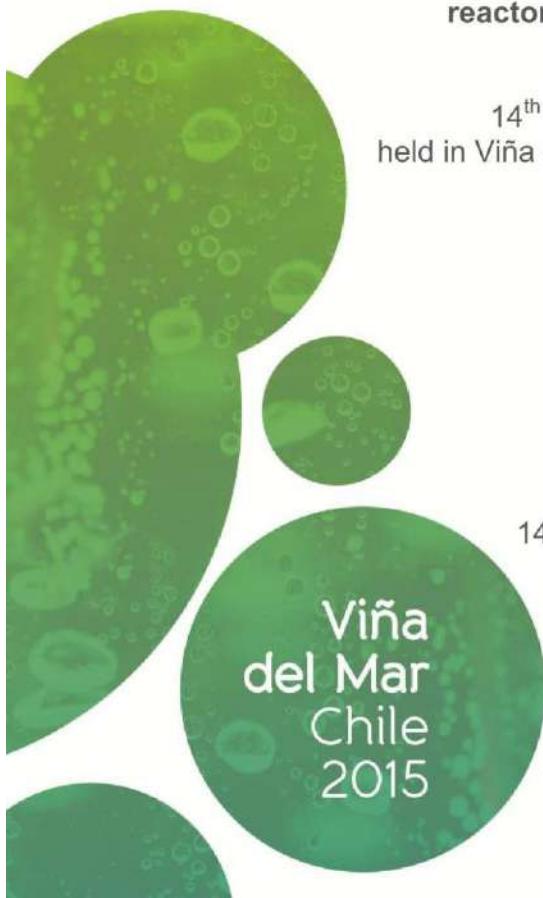
For the Poster Presentation named

**Influence of organic loading rate, source of inocula and support material on hydrogen production from sugarcane molasses in up-flow structured bed reactors operated under thermophilic condition**

In the  
14<sup>th</sup> World Congress on Anaerobic Digestion  
held in Viña del Mar, Chile, from November 15<sup>th</sup> to 18<sup>th</sup>, 2015.

**Dr. Rolando Chamy**  
Chairman

14th World Congress on Anaerobic Digestion





XII Latin American  
Workshop and  
Symposium on  
Anaerobic Digestion  
XII Taller y Simposio  
Latino Americano en  
Digestión Anaerobia

T12003

## CERTIFICADO

El XII DAAL otorga el presente certificado al trabajo:

**Hydrogen and Methane production from the sugarcane industry  
by-product by anaerobic bioreactors with fixed-structured bed  
(ABFSB) two-stage process operated under thermophilic condition**

Presentado en modalidad ORAL por los autores:

Rogerio Vilela  
Saia Flávia  
Langenhoff Alette  
Bas van der Zaan  
Márcia Zamariolli Damianovic

Cusco, 27 de Octubre del 2016

  
Carlos A. de Lemos Chernicharo  
Comité Científico

  
Jules van Lier  
Comité Científico

  
Rosa Eleria Yaya Beas  
Comité Organizador

  
Rosemary Vela de Cardich  
Comité Organizador



## CERTIFICADO

Certificamos que

**ROGÉRIO S. VILELA**

participou do

**II Seminário do Projeto Temático FAPESP**  
**"Produção de bioenergia no tratamento de**  
**água residuárias e adequação ambiental dos efluentes e resíduos gerados",**  
realizado no Instituto de Química de São Carlos da Universidade de São Paulo,  
nos dias 26 e 27 de julho de 2012.

A handwritten signature in black ink, appearing to read "Prof. Dr. Marcelo Zaiat".

Prof. Dr. Marcelo Zaiat  
Coordenador Geral do Projeto

A handwritten signature in black ink, appearing to read "Profa. Dra. Elisabete Moreira Assaf".

Profa. Dra. Elisabete Moreira Assaf  
Coordenadora Local

Oferecimento: Laboratório de Processos Biológicos (Programa de Pós-Graduação em Engenharia Hidráulica e Sanitária, Escola de Engenharia de São Carlos, Universidade de São Paulo); Laboratório de Eletroquímica e Catálise (Departamento de Físico-Química, Instituto de Química de São Carlos, Universidade de São Paulo); Laboratório de Engenharia Bioquímica (Escola de Engenharia Mauá, Instituto Mauá de Tecnologia); Laboratório de Controle Ambiental e de Simulação de Processos (Departamento de Controle e Processos, Universidade Federal de São Carlos); Laboratório de Tratamento de Águas e de Resíduos (Centro de Estudos Ambientais, Instituto de Geociências e Ciências Exatas, Universidade Estadual Paulista "Julio de Mesquita Filho").



## CERTIFICADO

Certificamos que o trabalho  
“Remoção de matéria orgânica de águas residuárias com elevada concentração de  
sulfato pelas vias sulfetogênica e metanogênica combinadas”

de autoria de  
Rogério S. Vitela, Márcia H. R. Damianovic e Eugenio Foresti  
foi apresentado no

**II Seminário do Projeto Temático FAPESP**  
**“Produção de bioenergia no tratamento de águas residuárias e  
adequação ambiental dos efluentes e resíduos gerados”**,  
realizado no Instituto de Química de São Carlos da Universidade de São Paulo,  
nos dias 26 e 27 de julho de 2012.

A handwritten signature in black ink, appearing to read 'Prof. Dr. Marcelo Zaiat'.

Prof. Dr. Marcelo Zaiat  
Coordenador Geral do Projeto

A handwritten signature in black ink, appearing to read 'Profa. Dra. Elisabete Moreira Assaf'.

Profa. Dra. Elisabete Moreira Assaf  
Coordenadora Local

Orientamento: Laboratório de Processos Biológicos (Programa de Pós-Graduação em Engenharia Hidráulica e Saneamento, Escola de Engenharia de São Carlos, Universidade de São Paulo); Laboratório de Eletroquímica e Catálise (Departamento de Físico-Química, Instituto de Química de São Carlos, Universidade de São Paulo); Laboratório de Engenharia Bioquímica (Escola de Engenharia Mauá, Instituto Mauá de Tecnologia); Laboratórios de Controle Ambiental e de Simulação de Processos (Departamento de Engenharia Química, Centro de Ciências Exatas e de Tecnologia, Universidade Federal de São Carlos); Laboratório de Tratamento de Águas e de Resíduos (Centro de Estudos Ambientais, Instituto de Geociências e Ciências Exatas, Universidade Estadual Paulista “Julio de Mesquita Filho”).

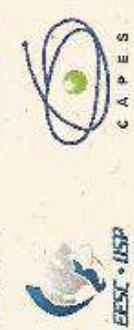
# CERTIFICADO

Certificamos que

Rogerio Silveira Vilela

participou do 1º Workshop Latino-Americano de Bio-Hidrogênio, realizado de 28 a 30 de julho de 2014, no The Palace Eventos, na cidade de São Carlos, SP, Brasil, com carga horária de 24 horas.

Realização:



São Carlos, 30 de julho de 2014.

Patrocinio:



Marcelo Zaiat

Vice-Coordenador do 1º WLABH<sub>2</sub>



Maria Bernadete A. Varesche  
Coordenadora do 1º WLABH<sub>2</sub>



# CERTIFICADO



Certificamos que o trabalho

Avaliação da geração de metano de água residuária rica em sulfato em reator anaeróbio horizontal de leito fixo – RAHLF

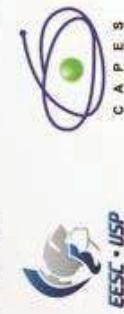
de autoria de

Rogerio Silveira Villela, Márcia Helena Rissatto Zamarianovic,  
Flávia Talarico Saia, Eugenio Foresti

foi apresentado no 1ºWorkshop Latino-American de Bio-Hidrogênio,  
realizado de 28 a 30 de julho de 2014, no The Palace Eventos,  
na cidade de São Carlos, SP, Brasil.

São Carlos, 30 de julho de 2014.

Realização:



Patrocínio:

  
**Marcelo Zaiat**  
Vice-Coordenador do 1º WLABH



**Maria Bernadete A. Varesche**  
Coordenadora do 1º WLABH<sub>2</sub>



## CERTIFICADO

Certificamos que

**Rogerio Silveira Vilela**

participou do V Seminário do Projeto Temático FAPESP “Produção de **Bioenergia no Tratamento de Águas Residuárias e Adequação Ambiental dos Efluentes e Resíduos Gerados**”, realizado na Escola de Engenharia de São Carlos, Universidade de São Paulo (EESC-USP), no dia 31 de Julho de 2015.

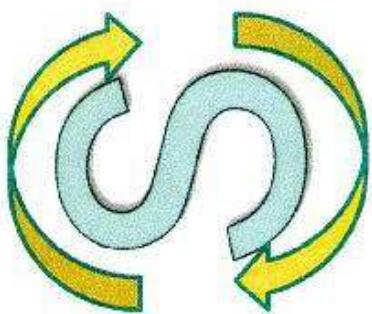
A handwritten signature in black ink, appearing to read 'Zaiat'.

Prof. Assoc. **Marcelo Zaiat (EESC-USP)**

Coordenador geral do projeto



# CERTIFICADO



Certificamos que

Rogerio Silveira Villela

participou do curso “**The Sulfur Cycle in Environmental Engineering**”,  
realizado entre os dias 06 e 08 de Julho de 2015 na  
Escola de Engenharia de São Carlos, Universidade de São Paulo (EESC/USP).

Profº Tit. Eugênio Foresti  
Coordenador do Curso



FUNDAÇÃO DE ENSINO  
SUPERIOR DE PASSOS



**FUNDAÇÃO DE ENSINO SUPERIOR DE PASSOS  
UNIVERSIDADE DO ESTADO DE MINAS GERAIS**

Criada pela Lei Estadual nº 2.933/63 - Estatuto aprovado pelo Decreto Estadual nº 16.998/75  
CNPJ 23.273.204/0001-00 - INSCRIÇÃO ESTADUAL - ISENTA

**C E R T I F I C A D O**

Certificamos, para os devidos fins, que o Biólogo Rogerio Silveira Vilela integrou na qualidade de membro a Comissão Examinadora da Banca de Exame do Trabalho de Conclusão de Curso intitulado de "**Impactos Ambientais Causados por Águas Residuárias de Abatedouro no Ribeirão Bocaina no Município de Passos – MG**", de Raquel Ferreira Machado, a uno da Faculdade de Engenharia de Passos (FEP), Fundação de Ensino Superior de Passos (FESP), Universidade do Estado de Minas Gerais (UEMG), realizado em 8 de dezembro de 2011. A comissão foi composta pelos seguintes membros: Profa. Dr. Odila Rigolin de Sá e Profa. Tânia Cristina Teles Oliveira.

Passos, 8 de dezembro de 2011.

Prof. Mancel Reginaldo Ferreira  
Diretor da Faculdade de Engenharia de Passos  
- FEP -

*Fesp, mais que educação, responsabilidade com o futuro*



**Universidade Federal de São Carlos  
Jornada de Iniciação Científica e Tecnológica**

**UFSCar**

Certificamos que o trabalho

**UTILIZAÇÃO DO ÍNDICE BMWP (UNQUEIRA E CAMPOS, 1998) EM TRÊS BRAÇOS DO RIO SAPUCAÍ NO  
RESERVATÓRIO DA UHE DE FURNAS - MG**

de autoria de

Rogerio Silveira Vilela, Nelci Lima Stripari e Kelly Carvalho

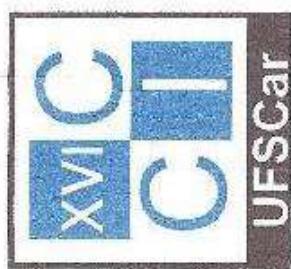
foi apresentado no XVI Congresso de Iniciação Científica durante a Jornada de Iniciação  
Científica e Tecnológica da UFSCar, de 7 a 10 de outubro de 2008.

São Carlos, 10 de outubro de 2008.

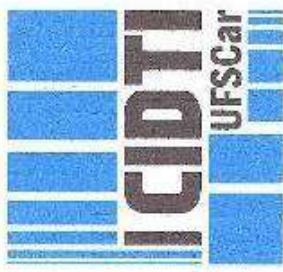
Prof. Dr. Joaquim de Araújo Nóbrega  
Coordenador de Iniciação Científica e Tecnológica

Prof. Dr. Claudio Shyinti Kiminami  
Pró-Reitor de Pesquisa

A declaração constante neste documento é de inteira responsabilidade do assinante, que se compromete a responder por sua autenticidade e integridade.



**Universidade Federal de São Carlos  
Jornada de Iniciação Científica e Tecnológica**



Certificamos que o trabalho

CARACTERIZAÇÃO DA COMUNIDADE BENTÔNICA EM TRÊS BRAÇOS DO RIO SAPUCAÍ NO  
RESERVATÓRIO NA UHE DE FURNAS - MG

de autoria de

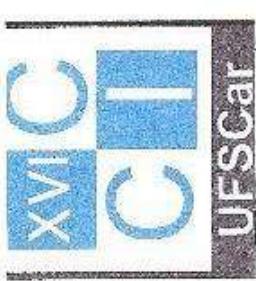
Rogerio Silveira Vilela, Nelci Lima Stripari, Kelly Carvalho e Lucas Rezende Penido  
Paschoal

foi apresentado no XVI Congresso de Iniciação Científica durante a Jornada de Iniciação  
Científica e Tecnológica da UFSCar, de 7 a 10 de outubro de 2008.

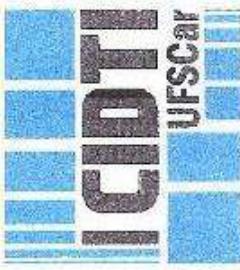
São Carlos, 10 de outubro de 2008.

Prof. Dr. Joaquim de Araújo Nóbrega  
Coordenador de Iniciação Científica e Tecnológica

Prof. Dr. Claudio Shyinti Kiminami  
Pró-Reitor de Pesquisa



Universidade Federal de São Carlos  
Jornada de Iniciação Científica e Tecnológica



Certificamos que o trabalho

AVALIAÇÃO DA DEGRAÇÃO DO RIO SÃO JOÃO EM ÁREAS AGROPASTORIS ATRAVÉS DA COMUNIDADE  
DE MACROINVERTEBRADOS BENTÔNICOS

de autoria de

Maria do Rosario Rodrigues Silva, Nelci Lima Stripari e Rogerio Silveira Villela

foi apresentado no XVI Congresso de Iniciação Científica durante a Jornada de Iniciação  
Científica e Tecnológica da UFSCar, de 7 a 10 de outubro de 2008.

São Carlos, 10 de outubro de 2008.

Prof. Dr. Joaquim de Araújo Nóbrega  
Coordenador de Iniciação Científica e Tecnológica

  
Prof. Dr. Claudio Shyinti Kiminami  
Pró-Reitor de Pesquisa

UNIVERSIDADE FEDERAL DE SÃO CARLOS  
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE  
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA E RECURSOS NATURAIS



**CERTIFICADO**

Certificamos que o trabalho "Avaliação da Qualidade da Água Utilizando o índice EWWP Junqueira e Campos, 1988 no Reservatório da UHE "Marechal Moraes" da Bacia Hidrográfica do Médio Rio Grande-MG", do autoria de ODILA RIGOLIN DE SÁ; Rogério Vilela; Karyna Cristilaine Pereira; Kelly de Carvalho; Michael Silveira Reis e Neuci de Lima Stripari, foi apresentado no II SIMPÓSIO DE ECOLOGIA DO PPQERN, realizado no "Anfiteatro Florestan Fernandes" da Universidade Federal de São Carlos - UFSCar, no período de 02 a 03 de outubro de 2008.

São Carlos, 03 de outubro de 2008.

Dra. Dalva Maria da S. Matos  
Coordenadora - PPQERN

Dra. Mirna Helena R. Seleg him  
Vice-Coordenadora-PPQERN



# Certificado



FURNAS

*Certificamos que ROGÉRIO SILVEIRA VILELA*

*participou do Encontro Técnico dos Pesquisadores Parceiros da Estação de  
Hidrobiologia e Piscicultura de Furnas, realizado nos dias 03 e 04 de  
setembro de 2007.*



FURNAS CIRASCI CORTA COM ISSA FONTE

*Furnas, 04 de setembro de 2007*

**Emílio José de Pádua Piantino**  
**Departamento de Produção Minas - D.R.M.O**

*Dra. Silvia Paiva*

**Vera da Silva Vieira Paiva**  
**Departamento de Engenharia Ambiental - DEAE**

[www.furnas.com.br](http://www.furnas.com.br)

A FESP/UEMG ATRAVÉS DA

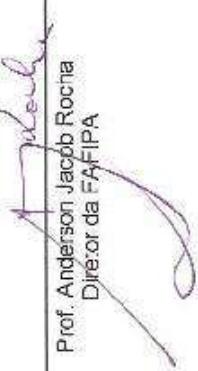
Faculdade de Filosofia de Passos

certifica que

Rogério Silveira Vilela

Proferiu a Palestra: "Ecotoxicologia e Ecologia de Reatores Anaeróbios" realizada dia 26/10/11  
durante o II Congresso de Ecologia do Sudoeste Mineiro.

  
Prof. Fábio Pimenta Esper Kallas  
Presidente do Conselho Curador

  
Prof. Anderson Jacob Rocha  
Diretor da FAFIPA

  
Prof. Odila Rigolin de Sa  
Coordenadora do Curso de CIÊNCIAS  
BIOLÓGICAS



**DECLARAÇÃO**

Declaro que o aluno **ROGÉRIO SILVEIRA VILELA**, atualmente graduado no curso de Ciências Biológicas, foi bolsista do PIBIC-FAPEMIG, registrado nesta coordenação, de março de 2007 à fevereiro de 2008, recebendo uma bolsa no valor de R\$300,00 mensais. O aluno participou do projeto “Avaliação da comunidade bentônica do Reservatório da UHE Marechal Macearenhas de Moraes na Bacia Hidrográfica do Médio Rio Grande-MG” sob orientação do Profa. Nelci de Lima Stripari.

Eduardo Goulart Collares

Coordenação de Pesquisa e Extensão da FESP

Data: Passos, 08 de julho de 2010.

# COMPROVAÇÕES – CURRICULUM VITAE ET STUDIORUM

73

Secretaria de Estado de Educação

Órgão Emissor: E.E. "Dr. Tancredo de Almeida Neves" - Município: Passos - Zona: Ufl  
Certidão de Contagem de Tempo

Certificamos, à vista de Livro de Ponto O exercício do

(fonte de dados da frequência)

Rogerio Silveira Vilela

(nome do servido)

(MASP/Conselho) DV

PEBR II A - Ciências

no(a)

(cargo atual / função)

**Escola Estadual "Dr. Tancredo de Almeida Neves"**, no período de 04/07/2007 a 12/07/2007  
(ano / período) (ano do último período)

sendo conforme Grade de Freqüência: 9 dias de efetivo exercício x 9 dias de férias prêmio x  
dias de licença-maternidade / paternidade; x dias de licença / afastamento para tratamento de saúde; x  
dias de faltas abonadas / anistiadas; x dias de Auxílio-Doença, com vínculo empregatício; x dias de  
afastamento não remunerado com contribuição preventiva; x dias de faltas.

ANO	Ocorrência	Grade de Freqüência												Cargo/Função	Regente	Período	Sf. Func. (classe/desig.)
		Janeiro	Fevereiro	Março	Abri	Mai	Junho	Julho	Agosto	Setembro	Outubro	Novembro	Dezembro				
2007	Efec. Exerc.	-	-	-	-	-	-	-	-	-	-	-	-	PEBR II A Ciências 18 A/S	TD: 04/07/07 a 12/07/2007		
	Lic. Mater/Pater	-	-	-	-	-	-	-	-	-	-	-	-				
	Lic. Saúde	-	-	-	-	-	-	-	-	-	-	-	-				
	Férias Prêmio	-	-	-	-	-	-	-	-	-	-	-	-				
	Faltas	-	-	-	-	-	-	-	-	-	-	-	-				
	Afas. não remun.	-	-	-	-	-	-	-	-	-	-	-	-				
Obs:																	

ANO	Ocorrência	Grade de Freqüência												Cargo/Função	Regente	Período	Sf. Func. (classe/desig.)
		Janeiro	Fevereiro	Março	Abri	Mai	Junho	Julho	Agosto	Setembro	Outubro	Novembro	Dezembro				
2007	Efec. Exerc.	-	-	-	-	-	-	-	-	-	-	-	-				
	Lic. Mater/Pater	-	-	-	-	-	-	-	-	-	-	-	-				
	Lic. Saúde	-	-	-	-	-	-	-	-	-	-	-	-				
	Férias Prêmio	-	-	-	-	-	-	-	-	-	-	-	-				
	Faltas	-	-	-	-	-	-	-	-	-	-	-	-				
	Afas. não remun.	-	-	-	-	-	-	-	-	-	-	-	-				
Obs:																	

ANO	Ocorrência	Grade de Freqüência												Cargo/Função	Função exercida	Período	Sf. Func. (classe/desig.)
		Janeiro	Fevereiro	Março	Abri	Mai	Junho	Julho	Agosto	Setembro	Outubro	Novembro	Dezembro				
2007	Efec. Exerc.	-	-	-	-	-	-	-	-	-	-	-	-				
	Lic. Mater/Pater	-	-	-	-	-	-	-	-	-	-	-	-				
	Lic. Saúde	-	-	-	-	-	-	-	-	-	-	-	-				
	Férias Prêmio	-	-	-	-	-	-	-	-	-	-	-	-				
	Faltas	-	-	-	-	-	-	-	-	-	-	-	-				
	Afas. não remun.	-	-	-	-	-	-	-	-	-	-	-	-				
Obs:																	

Passos, 9 de abril de 2008

Responsável: RB (assinatura)

390.054-5

MASP/EN

Diretor: \_\_\_\_\_

Reis Paulino de Andrade  
Diretor MG 03/07/2007  
Pag. 38, col. 04

**INSPETOR**  
**Lucy Vidalgal**  
Inspetor Escolar  
Msn. 1 1107-5  
Reg. 3604442



**MUNICÍPIO DE PASSOS**  
PREFEITURA MUNICIPAL  
ESTADO DE MINAS GERAIS

**DECLARAÇÃO**

Declaro para os devidos fins que Rogério Silveira Vilela, aluno da FESP no curso Ciências Biológicas, esteve estagiando (20 horas semanais) na Jornada Ampliada Novo Horizonte atuando na área de reforço com crianças de 07 à 15 anos.

Data de início: 28/02/2005.

Data de término: 24/02/2006.

Na verdade firmo a presente.

  
**Carla A. S. Pimente Caixeta de Melo**  
Diretora de Assistência Social



Universidade Federal de Minas Gerais  
ICB, Depto. Biologia Geral, Lab. Ecologia da Biotica  
Av. Antonio Carlos, 6627, CP. 483, CEP. 31.270-901  
Belo Horizonte, MG. [www.icb.ufmg.br/diy/berthos](http://www.icb.ufmg.br/diy/berthos), [callisto@icb.ufmg.br](mailto:callisto@icb.ufmg.br), [callistom@ufmg.br](mailto:callistom@ufmg.br)



## **DECLARAÇÃO**

Declaro, para os devidos fins, que Rogério Silveira Vilela realizou estagiário voluntário no Laboratório NUVELHAS/Projeto Manuelzão/UFMG de 11 a 22/08 com carga Horária de 80 hs, sob minha orientação.

Belo Horizonte, 27 de agosto de 2008.

Prof. Dr. Marcos Callisto  
UFMG

**CERTIFICADO DE PROFICIÊNCIA**



Test of English for Academic Purposes

Nome: Rogerio Silveira Vilcia

Nº Documento:	042248.716-37
Data do Exame:	21/10/2011
Código de Identificação:	56341
Local do Exame:	São Carlos - SP



Pontuação obtida no Exame de Proficiência  
em Leitura de Textos em Inglês TEAP  
na área de **Exatas/Tecnológicas**:

**87** ( Oitenta e sete )

Informações importantes para as instituições que receberam este certificado.

- A taxa de aprovação é de 25% de 100 candidatos.
- As informações estão armazenadas no banco de dados da TCSCT.
- Recomenda-se a bordagem da bora em brancas no certificado original e que seja válido se tratar a chancela autorizada.
- A validade é comunicada para os resultados obtidos 60 dias após.

**EFESEPrime**  
Sistema de ensino integral.  
[www.teapprime.org](http://www.teapprime.org)  
[carlaldo@teapprime.org](mailto:carlaldo@teapprime.org)

Prof. Dr. Heitor de Paula Carvalho  
Dirigente de Exames



**FUNDAÇÃO DE ENSINO SUPERIOR DE PASSOS  
UNIVERSIDADE DO ESTADO DE MINAS GERAIS**

Criada pela Lei Estadual nº 2.933/63 - Estatuto aprovado pelo Decreto Estadual nº 16.998/73  
CNPJ 23.273.204/0001-00 - INSC. ESTADUAL - ISENTE

# CERTIFICADO

Certificamos que

**Rogério Silveira Vilela**

participou do mini-curso ministrado pelo Professor Luiz Henrique Anzaloni Pedrosa, "**Biologia, Comportamento e Extração de Veneno de Serpentes**", durante a Semana Universitária da Faculdade de Filosofia de Passos.

Passos, maio de 2007.

**Odila Rigolin de Sá**  
*Coordenadora de C. Biológicas*

**Eunice Blanco Pereira Lima**  
*Diretora da FAHFA*

A presença ao evento equivale a 4 horas de Estágio Supervisionado.



**FUNDAÇÃO DE ENSINO SUPERIOR DE PASSOS  
UNIVERSIDADE DO ESTADO DE MINAS GERAIS**

Criada por a Lei Estadual nº 2.933/63. Estatuto aprovado pelo Decreto Estadual nº 16.998/75.  
CNPJ 23.273.204/0001-00 - INSC. LSTATUAL - ISLNIA.

# CERTIFICADO

Certificamos que, **Rogério Silveira Villela**, participou do mini-curso ministrado pelos Biólogos Marcos Barreto e João Paraíso, “**Ecotoxicidade de Organismos de Água-doce**”, durante a Semana Universitária da Faculdade de Filosofia de Passos.

Passos, agosto de 2006.

Odila Rigolin de Sá  
COORDENADORA DE CIÊNCIAS BIOLÓGICAS

Eunice Blanco Pereira Lima  
DIRETORA DA FAFIPA

A presença ao evento equivale a 20 horas de Estágio Supervisionado



**FUNDAÇÃO DE ENSINO SUPERIOR DE PASSOS  
UNIVERSIDADE DO ESTADO DE MINAS GERAIS**

Crada pela Lei Estadual nº 2.933/63 - Estatuto aprovado pelo Decreto Estadual nº 16.998/75  
CNPJ 23.273.204/0001-00 - INSC. ESTADUAL - ISENTE.

# CERTIFICADO

Certificamos que, **Rogério Silveira Vilela**, participou do mini-curso, “**Teste de Toxicidade em *Daphnia Similis***”, durante a Semana Universitária da Faculdade de Filosofia de Passos.

Passos, agosto de 2006.

Odila Rigolin de Sá  
COORDENADORA DE CIÊNCIAS BIOLÓGICAS

Eunice Blanco Pereira Lima  
DIRETORA DA FAFIPA

A presença ao evento equivale a 20 horas de Estágio Supervisionado



**FUNDAÇÃO DE ENSINO SUPERIOR DE PASSOS  
UNIVERSIDADE DO ESTADO DE MINAS GERAIS**

Criada por a Lei Estadual nº 2.933/63. Estatuto aprovado pelo Decreto Estadual nº 16.998/75  
CNPJ 23.273.204/0001-00 - INSC. ESTADUAL - ISENTA

# **CERTIFICADO**

Certificamos que, **Rogério Silveira Vilela**, participou do mini-curso ministrado pela Dra. Mara Adriana Marçal Simabuku, “**Alimentação Natural de Peixes**”, durante a Semana Universitária da Faculdade de Filosofia de Passos.

Passos, agosto de 2006.

Odila Rigolin de Sá  
*COORDENADORA DE CIÊNCIAS BIOLÓGICAS*

Eunice Blanco Pereira Lima  
*DIRETORA DA FAFIPA*

A presença ao evento equivale a 20 horas de Estágio Supervisionado



**FUNDAÇÃO DE ENSINO SUPERIOR DE PASSOS  
UNIVERSIDADE DO ESTADO DE MINAS GERAIS**

Criada pela Lei Estadual nº 2.933/63 - Estatuto aprovado pelo Decreto Estadual nº 16.998/75  
CNPJ 23.273.204/0001-00 - INSC. ESTADUAL - ISPFNTA

# CERTIFICADO

Certificamos que, **Rogério Silveira Vilela**, participou do mini-curso ministrado pela Dra. Mariluce Gonçalves Fonseca, “**Biologia e Reconhecimento de Serpentes Peçonhentas e Não Peçonhentas**”, durante a Semana Universitária da Faculdade de Filosofia de Passos.

Passos, agosto de 2006.

Odila Rigolin de Sá  
COORDENADORA DE CIÊNCIAS BIOLÓGICAS

Eunice Blanco Pereira Lima  
DIRETORA DA FAFIPA

A presença ao evento equivale a 20 horas de Estágio Supervisionado



**FUNDAÇÃO DE ENSINO SUPERIOR DE PASSOS  
UNIVERSIDADE DO ESTADO DE MINAS GERAIS**

Criada pela Lei Estadual nº 2.933/63 - Estatuto aprovado pelo Decreto Estadual nº 16.903/75.  
CNPJ 25.273.204/0001-00 - INSC. ESTADUAL - ISENTE

# CERTIFICADO

Certificamos que, **Rogério Silveira Vilela**, participou do mini-curso ministrado pelo Msc. José de Paula Silva, "Macro-Fotografia", durante a Semana Universitária da Faculdade de Filosofia de Passos.

Passos, agosto de 2006.

Odila Rigolin de Sá  
COORDENADORA DE CIÊNCIAS BIOLÓGICAS

Eunice Blanco Pereira Lima  
DIRETORA DA FAFIPA

A presença ao evento equivale a 20 horas de Estágio Supervisionado



**FUNDAÇÃO DE ENSINO SUPERIOR DE PASSOS  
UNIVERSIDADE DO ESTADO DE MINAS GERAIS**

Criada pela Lei Estadual nº 2.933/63 - Estatuto aprovado pelo Decreto Estadual nº 16.993/75  
CNPJ 23.273.204/0001-00 - INSC. ESTADUAL - ISENTE

# **CERTIFICADO**

Certificamos que, **Rogério Silveira Vilela**, participou do mini-curso ministrado pelo Técnico Agrícola José Benedito da Silva de Furnas Centrais Elétricas, “**Produção de Mudas: Reflorestamento de Mata Ciliar**”, durante a Semana Universitária da Faculdade de Filosofia de Passos.

Passos, agosto de 2006.

Odila Rigolin de Sá  
COORDENADORA DE CIÊNCIAS BIOLÓGICAS

Eunice Blanco Pereira Lima  
DIRETORA DA FAFIPA

A presença ao evento equivale a 20 horas de Estágio Supervisionado

# CERTIFICADO

Certificamos que, **Rogério Silveira Vilela**, participou do mini-curso ministrado pelos Biólogos Marcos Barreto e João Paraíso, “**Teste de Toxicidade em Ouriço-do-mar**”, durante a Semana Universitária da Faculdade de Filosofia de Passos.

Passos, agosto de 2006.

Odila Rigolin de Sá  
COORDENADORA DE CIÉNCIAS BIOLÓGICAS

Eunice Blanco Pereira Lima  
DIRETORA DA FAFIPA

A presença ao evento equivale a 20 horas de Estágio Supervisionado

# CERTIFICADO

Certificamos que, **Rogério Silveira Vilela**, participou do mini-curso, “**Reprodução Induzida em Peixes**”, durante a Semana Universitária da Faculdade de Filosofia de Passos.

Passos, agosto de 2006.



Odila Rigolin de Sá  
COORDENADORA DE CIÊNCIAS BIOLÓGICAS



Eunice Blanco Pereira Lima  
DIRETORA DA FAFIPA

A presença ao evento equivale a 20 horas de Estágio Supervisionado