Universidade Estadual de Londrina

RODRIGO JOSÉ GOMES

PRODUÇÃO DE BIOCELULOSE POR BACTÉRIAS ACÉTICAS UTILIZANDO TÉCNICAS DE OTIMIZAÇÃO NUTRICIONAL E DE CULTIVO

Londrina
2021

RODRIGO JOSÉ GOMES

PRODUÇÃO DE BIOCELULOSE POR BACTÉRIAS ACÉTICAS UTILIZANDO TÉCNICAS DE OTIMIZAÇÃO NUTRICIONAL E DE CULTIVO

Tese apresentada ao Programa de Pós-Graduação em Ciência de Alimentos, nível Doutorado, da Universidade Estadual de Londrina, como requisito parcial à obtenção do título de Doutor em Ciência de Alimentos.

Orientadora: Dra. Wilma Aparecida Spinosa

Coorientadora: Dra. Elza Iouko Ida

Londrina
2021

Ficha de identificação da obra elaborada pelo autor, através do Programa de Geração Automática do Sistema de Bibliotecas da UEL

Gomes, Rodrigo José.
Produção de biocelulose por bactérias acéticas utilizando técnicas de otimização nutricional e de cultivo / Rodrigo José Gomes. - Londrina, 2021.
190 f. : il.

Orientador: Wilma Aparecida Spinosa.
Coorientador: Elza Iouko Ida.
Tese (Doutorado em Ciência de Alimentos) - Universidade Estadual de Londrina, Centro de Ciências Agrárias, Programa de Pós-Graduação em Ciência de Alimentos, 2021.
Inclui bibliografia.

1. Fermentação acética - Tese. 2. Nutrição microbiana - Tese. 3. Biopolímero - Tese. 4. Nutrientes - Tese. I. Spinosa, Wilma Aparecida . II. Ida, Elza Iouko. III. Universidade Estadual de Londrina. Centro de Ciências Agrárias. Programa de Pós-Graduação em Ciência de Alimentos. IV. Título.

CDU 641.1

RODRIGO JOSÉ GOMES

PRODUÇÃO DE BIOCELULOSE POR BACTÉRIAS ACÉTICAS UTILIZANDO TÉCNICAS DE OTIMIZAÇÃO NUTRICIONAL E DE CULTIVO

Tese apresentada ao Programa de Pós-Graduação em Ciência de Alimentos, nível doutorado, da Universidade Estadual de Londrina, como requisito parcial à obtenção do título de Doutor em Ciência de Alimentos.

BANCA EXAMINADORA

_____
Orientadora: Profa. Dra. Wilma Aparecida Spinosa
Universidade Estadual de Londrina - UEL

_____
Prof. Dr. Edson Marcelino Alves
Universidade Federal do Triângulo Mineiro - UFTM

_____
Prof. Dr. Leonel Vinícius Constantino
Universidade Estadual de Londrina - UEL

_____
Profa. Dra. Morsyleide de Freitas Rosa
Embrapa Agroindústria Tropical

_____
Prof. Dr. Pedro de Oliva Neto
Universidade Estadual Paulista - UNESP

Londrina, 25 de novembro de 2021.

Dedico este trabalho aos meus pais Angela e José, à minha irmã Janaina e aos sobrinhos Pedro e Théo.
Todo o meu amor a vocês.

AGRADECIMENTOS

A Deus, por me dar saúde física e mental durante todo o período de execução deste trabalho.

À Profª. Drª. Wilma Spinosa, pela orientação, generosidade, confiança e amizade de sempre.

À Profª. Drª. Elza Iouko Ida, pela co-orientação, revisão e sugestões ao trabalho final.

A todos os docentes e ténicos dos laboratórios do departamento de Ciência e Tecnologia de Alimentos da UEL.

Ao Laboratório de Espectroscopia – ESPEC da Pró Reitoria de Pesquisa e Pós Graduação da UEL, em especial às técnicas Natália e Aline, pela disponibilidade para realização das análises de FT-IR, TG e DSC.

Ao Laboratório de Difração de Raios X – LARX da Pró Reitoria de Pesquisa e Pós Graduação da UEL, em especial ao Prof. Dr. Paulo Rogério Catarini da Silva, pela disponibilidade para realização das análises de DRX.

Ao CNPq, pelo fornecimento de bolsa de estudo, sendo esta de fundamental importância para a execução e continuidade do trabalho.

À Embrapa Tropical, pelo fornecimento de materiais utilizados na pesquisa.

À minha família e a todos que de alguma forma contribuíram e estiveram presentes com palavras de incentivo, amizade e carinho durante este período, em especial aos meus pais Agela e José, à minha irmã Janaina e a todos os alunos e amigos do grupo de pesquisa coordenado pela profa. Wilma Spinosa.

GOMES, Rodrigo José. **Produção de biocelulose por bactérias acéticas utilizando técnicas de otimização nutricional e de cultivo**. 2021. 190 f. Tese (Doutorado em Ciência de Alimentos) – Universidade Estadual de Londrina, Londrina, 2021.

RESUMO

As bactérias do ácido acético (BAA) compreendem uma variedade de microrganismos que são utilizados para obtenção de diversos produtos, entre eles a biocelulose. O estudo dos efeitos da suplementação de nutrientes no meio de cultura bem como o aprimoramento das técnicas de cultivo são importantes etapas que antecedem a produção e aplicação destes compostos, pois podem melhorar o rendimento, a produtividade e as características do biomaterial obtido. Desta forma, este trabalho objetivou avaliar os requerimentos nutricionais de BAA, em nível qualitativo e quantitativo, e seu impacto na produção e propriedades da biocelulose produzida. Para isto, foram avaliados os efeitos da suplementação do meio de cultura com diversos minerais (boro, ferro, manganês, molibdênio e zinco), vitaminas (tiamina - B_1, riboflavina - B_2, ácido nicotínico - B_3, ácido pantotênico - B_5, piridoxina - B_6, biotina - B_7, ácido fólico - B_9, cianocobalamina - B_{12}, ácido *p*-aminobenzóico e *myo*-inositol), e aminoácidos (ácido aspártico, ácido glutâmico, alanina, arginina, asparagina, cisteína, fenilalanina, glicina, glutamina, histidina, isoleucina, leucina, lisina, metionina, prolina, serina, treonina, triptofano e valina) através de planejamento fatorial de Plackett & Burman. Os fatores com efeito positivo mais significativo foram selecionados para otimização através de planejamento composto central. Além disso, foi avaliado o efeito do tempo de pré-cultivo de BAA (35 dias ou 56 dias) na produtividade e nas características da biocelulose obtida após fermentação de 10 dias. As membranas produzidas nas condições ótimas foram caracterizadas através de análises de espectroscopia de infravermelho, difração de raios-X, termogravimetria, calorimetria exploratória diferencial, e capacidades de retenção e reidratação de água para determinar suas características e propriedades. Entre os minerais, o ferro, o manganês e o zinco foram requeridos por duas linhagens (*Komagataeibacter hansenii* ATCC 23769 e *Komagataeibacter intermedius* V-05) enquanto que as vitaminas ácido pantotênico e ácido nicotínico foram as que tiveram o efeito positivo mais significativo para as duas espécies. Riboflavina e tiamina tiveram efeito negativo significativo e foram consideradas fatores de diminuição da produção de biocelulose. A produção alcançada nas condições ótimas para os fatores selecionados foi de 1,35 g L^{-1} pela espécie *K. hansenii* ATCC 23769 e 2,23 g L^{-1} pela espécie *K. intermedius* V-05. Entre os aminoácidos, ácido aspártico, fenilalanina e serina foram requeridos pela espécie *K. intermedius* V-05. Os aminoácidos apolares (alanina, leucina e triptofano), sulfurados (cisteína e metionina) e carregados positivamente (arginina e histidina) tiveram efeito negativo significativo e foram considerados fatores de diminuição da produção de biocelulose.

A produção alcançada nas condições ótimas para os fatores selecionados foi de 3,05 g L^{-1}. As membranas obtidas nas condições otimizadas pela espécie *K. hansenii* ATCC 23769 apresentaram melhora no rendimento, cristalinidade e propriedades térmicas, enquanto que as membranas produzidas pela espécie *K. intermedius* V-05 apresentaram melhora nas propriedades térmicas e hidrofílicas. O tempo de pré-cultivo de 56 dias antes da fermentação também impactou na produtividade pela espécie *K. hansenii* ATCC 23769, alcançando 1,72 g L^{-1} ou 187% de aumento em relação a cepa não pré-cultivada, sendo também observado uma melhora na cristalinidade e nas propriedades térmicas das amostras. Com este trabalho, alcançou-se um melhor entendimento sobre os fatores que afetam a produção de biocelulose por bactérias acéticas, os quais também impactam nas características e propriedades das membranas produzidas.

Palavras-chave: Fermentação acética. Nutrição microbiana. Nutrientes minerais. Vitaminas. Aminoácidos. Biopolímero.

GOMES, Rodrigo José. **Bacterial cellulose production by acetic acid bacteria using methods of nutritional optimization and cultivation**. 2021. 190 f. Tese (Doutorado em Ciência de Alimentos) – Universidade Estadual de Londrina, Londrina, 2021.

ABSTRACT

Acetic acid bacteria (AAB) comprise a variety of microorganisms that are used to obtain several biotechnological products, such as bacterial cellulose (BC). Studying the effects of supplementation with nutrients in culture medium, as well as the improvement of cultivation techniques are important steps that precede the production and application of biotechnological compounds. Through this, it can be improved yield, productivity and characteristics of the biomaterials. Thus, this work aimed to evaluate the nutritional requirements of AAB, at qualitative and quantitative level, and its impact on the production and properties of produced BC. For this, it was evaluated the effects of supplementing the culture medium with several minerals (boron, iron, manganese, molybdenum and zinc), vitamins (thiamine - B_1, riboflavin - B_2, nicotinic acid - B_3, pantothenic acid - B_5, pyridoxine - B_6, biotin - B_7, folic acid - B_9, cyanocobalamin - B_{12}, *p*-aminobenzoic acid and *myo*-inositol), and amino acids (alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan and valine) using Plackett & Burman factorial design. Factors with most significant positive effect were selected for optimization through central composite design. Furthermore, the effect of pre-cultivation time (35 days or 56 days) on the productivity and characteristics of the BC obtained after the 10-day fermentation process was evaluated. Membranes produced at optimal conditions were characterized by infrared spectroscopy, X-ray diffraction, thermogravimetry, differential scanning calorimetry, water holding capacity and rehydration capacity to be determined their characteristics and properties. Among minerals, iron, manganese and zinc were required by two strains (*Komagataeibacter hansenii* ATCC 23769 and *Komagataeibacter intermedius* V-05) while vitamins pantothenic acid and nicotinic acid had the most significant positive effect for the same strains. Riboflavin and thiamine had a significant negative effect and were considered to decrease BC biosynthesis. The production at optimal conditions for selected micronutrients reached 1.35 g L^{-1} by the strain *K. hansenii* ATCC 23769 and 2.23 g L^{-1} by the strain *K. intermedius* V-05. Among amino acids, aspartic acid, phenylalanine and serine were required by the strain *K. intermedius* V-05. Nonpolar (alanine, leucine and tryptophan), containing sulfur (cysteine and methionine) and positively charged (arginine and histidine) amino acids had a significant negative effect and were considered to decrease BC biosynthesis. The production at optimal conditions for selected amino acids reached 3.05 g L^{-1}. Membranes produced under optimal conditions by the strain *K. hansenii* ATCC 23769 had an improvement in yield, crystallinity and thermal properties, while the membranes produced by the strain

K. intermedius V-05 had an improvement in thermal and hydrophilic properties. The 56-day pre-cultivation time before fermentation also impacted the productivity of the strain *K. hansenii* ATCC 23769, reaching 1.72 g L^{-1} or 187% increase in relation to the non-pre-cultivated strain also improving crystallinity and thermal properties of the samples. By this work, it was achieved a better understanding of the factors that affect the production of BC by AAB strains, which also influence the characteristics and properties of the produced samples.

Key Words: Acetic fermentation. Microbial nutrition. Mineral nutrients. Vitamins. Amino acids. Biopolymer.

LISTA DE ILUSTRAÇÕES

CAPÍTULO I

CAPÍTULO II

CAPÍTULO III

CAPÍTULO IV

APÊNDICES

LISTA DE TABELAS E QUADROS

CAPÍTULO I

CAPÍTULO II

CAPÍTULO III

CAPÍTULO IV

APÊNDICES

LISTA DE ABREVIATURAS E SIGLAS

AAB	Acetic Acid Bacteria
ADH	Alcohol Dehydrogenase / Álcool Desidrogenase
ALDH	Aldehyde Dehydrogenase / Aldeído Desidrogenase
ANOVA	Análise de Variância
ATP	Adenosine Triphosphate
BAA	Bactérias do Ácido Acético
BC	Bacterial Cellulose
Bcs	Bacterial cellulose synthase / Celulose bacteriana sintase
CCD	Central Composite Design
cdi-GMP	cyclic dimeric guanosine monophosphate / diguanilmonofosfato cíclico
CFU	Colony Forming Units
CoA	Coenzyme A
CrI	Crystallinity Index
DSC	Differential Scanning Calorimetry
DTG	Derivative Thermogravimetry
EMS	Ethyl Methane Sulfonate
FAD	Flavin Adenine Dinucleotide
FMN	Flavin Mononucleotide
FTIR	Fourier Transform Infrared Spectroscopy
GDH	Glucose Dehydrogenase
HS	Hestrin-Schramm
MEV	Microscopia Eletrônica de Varredura
MSR	Metodologia de Superfície de Resposta
MYP	Mannitol-Yeast exract-Peptone
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PQQ	Pyrroloquinoline Quinone
PS	Phosphatidylserine
PVDF	Polyvinylidene Difluoride
RBP	Riboflavin Biosynthetic Pathway

RC	Rehydration Capacity
RSM	Response Surface Methodology
SE	Standard Error
TCA	Tricarboxylic Acid
TG	Thermogravimetry
TGA	Thermogravimetric Analysis
UDP	Uridine Diphosphate / Uridina difosfato
UTP	uridine Triphosphate
UV	Ultraviolet
WHC	Water Holding Capacity
XRD	X-Ray Diffraction

SUMÁRIO

1. INTRODUÇÃO

A celulose é o biopolímero mais abundante do planeta com produção natural de bilhões de toneladas por ano. Têm sido considerada como um dos recursos naturais mais importantes, sendo amplamente utilizada na indústria devido à sua disponibilidade, elevada biodegradabilidade e renovabilidade. A celulose forma a base estrutural das paredes celulares em plantas e algas eucarióticas e também pode ser sintetizada por microrganismos (HUANG et al, 2011; YANG et al., 2013). No entanto, as plantas contêm celulose em combinação com outros compostos, como lignina, pectina e hemicelulose. Devido a esta combinação, a purificação da celulose demanda mais energia, água e processos químicos intensivos que resultam em mudanças irreversíveis na sua estrutura. O aumento da produção de celulose a partir de fonte vegetal também acarreta em problemas ambientais como: esgotamento de reservas florestais e água; poluição do ar, da água e do solo devido à geração de efluentes tóxicos das indústrias; e consumo de energia a partir de combustíveis fósseis (TYAGI; SURESH, 2016).

Em contrapartida, a produção microbiológica de celulose pode representar uma opção para determinados nichos cujo produto final tem maior valor agregado. Por isso, ela tem atraído interesses de pesquisadores nos últimos anos, também devido às suas propriedades e características consideradas incomuns, tais como, elevada pureza, alto grau de polimerização, porosidade, cristalinidade, resistência mecânica e biocompatibilidade, consistindo em uma rede tridimensional de fibras nanoestruturada. Após a década de 1990, diversas pesquisas focalizaram na sua produção, características estruturais, propriedades e aplicações. No período entre 2009 a 2019, o número de publicações sobre celulose bacteriana aumentou mais de 200% e correspondeu a um total de 59.158 publicações nestes 10 anos (FERNANDES et al., 2020; MOHITE; PATIL, 2014). Destaca-se que no período entre 1954 a 2020, o Brasil ocupou o 3°lugar no número de publicações relacionadas a meios de cultivo para produção de biocelulose, sendo em 1°lugar a China, 2°lugar os Estados Unidos, 4ºlugar o Japão e seguidos da Índia e Coreia do Sul, respectivamente (SPEROTTO et al., 2021).

A biocelulose tem um grande potencial de aplicação nas indústrias de alimentos, fármacos, papel, materiais cicatrizantes etc. No entanto, apesar de sua versatilidade, o baixo rendimento da produção tem limitado amplamente as suas

aplicações. Assim, a melhoria da tecnologia atual e o desenvolvimento de novos métodos são essenciais e extremamente necessários para aumentar o rendimento da produção. Entre estas abordagens, estão: (i) a seleção de linhagens com alto rendimento de produção, como a espécie *Komagataeibacter intermedius* V-05, isolada a partir de indústria de vinagre e que apresentou alto rendimento de produção em um meio a base de melaço de soja desenvolvido em nosso trabalho de pesquisa anterior (GOMES et al., 2021); (ii) a seleção de métodos de cultivo eficientes; e (iii) a otimização da composição dos meios de cultura (LU et al., 2011). Dentro desta perspectiva, novos métodos de produção têm sido desenvolvidos com o objetivo de não só melhorar o rendimento, mas também a estrutura e outras propriedades físicas das membranas para aplicações específicas (GEA et al., 2011).

Os microrganismos possuem necessidades nutricionais distintas e os fatores nutricionais, as condições físico-químicas e as concentrações dos componentes do meio são parâmetros que desempenham um papel significativo no crescimento microbiano e na síntese dos metabólitos. Entre os fatores nutricionais requeridos estão as fontes de carbono, fontes de nitrogênio tais como os aminoácidos e os micronutrientes (minerais e vitaminas). Para a catálise de certas reações, por exemplo, as enzimas frequentemente requerem a participação de cofatores que incluem íons metálicos e vitaminas. Os aminoácidos são os blocos de construção para formação de diversas enzimas e proteínas de organismos vivos (ANBU; ANNADURAI; HUR, 2013; COCHRANE; STROBEL, 2008; ESWARI; ANAND; VENKATESWARLU, 2016).

Com relação aos requerimentos nutricionais de bactérias do ácido acético, o enfoque na produção de biocelulose ainda tem sido pouco explorado. Assim sendo, a investigação dos parâmetros nutricionais destes microrganismos que apresentam potencial de aplicação industrial, será relevante e importante para que estes nutrientes sejam fornecidos adequadamente e para melhorar o seu processo da produção e produtividade.

A otimização dos parâmetros nutricionais de microrganismos pode ser realizada por meio de técnicas estatísticas, que podem apresentar diversas vantagens em comparação aos métodos convencionais (PRAJAPATI; TRIVEDI; PATEL, 2013). Em biotecnologia, a metodologia de superfície de resposta tem sido aplicada para obtenção de uma ampla gama de metabólitos microbianos primários e secundários, e tem se mostrado uma ferramenta experimental eficiente na qual

podem ser determinadas as condições ótimas de um sistema multivariado (BHARGAVI; PRAKASHAM, 2016; VAIDYA et al., 2009). Ainda, o aperfeiçoamento dos métodos de cultivo pode resultar em melhorias que podem impactar na economia e no rendimento do processo.

Considerando o exposto, esta pesquisa visa investigar diversos fatores que influenciam na produção e as características da biocelulose por bactérias acéticas do gênero *Komagataeibacter* que podem viabilizar a produção em larga escala e aplicação industrial deste biopolímero.

2. OBJETIVOS

2.1. OBJETIVO GERAL

Investigar diversos fatores que potencialmente influenciam a produção e características da biocelulose por bactérias acéticas do gênero *Komagataeibacter*.

2.2. OBJETIVOS ESPECÍFICOS

- Avaliar o efeito da suplementação qualitativa de minerais, vitaminas e aminoácidos na produção de biocelulose pelas espécies *Komagataeibacter hansenii* ou *Komagataeibacter intermedius*.
- Investigar o efeito da concentração de minerais, vitaminas e aminoácidos selecionados sobre a produção de biocelulose por espécies de *Komagataeibacter hansenii* ou *Komagataeibacter intermedius*.
- Avaliar a influência do tempo de pré-cultivo na produção de celulose bacteriana e as características e propriedades das celuloses obtidas.

3. REFERÊNCIAS

ANBU, P.; ANNADURAI, G.; HUR, B. K. Production of alkaline protease from a newly isolated *Exiguobacterium profundum* BK-P23 evaluated using the response surface methodology. **Biologia,** v. 68, n. 2, p. 186-193, abr. 2013. https://doi.org/10.2478/s11756-013-0159-5

BHARGAVI, P. L.; PRAKASHAM, R. S. Enhanced fibrinolytic protease production by *Serratia marcescens* RSPB11 through Plackett-Burman and response surface methodological approaches. **Journal of Applied Biology & Biotechnology,** v. 4, n. 3, p. 6-14, maio/jun. 2016. https://doi.org/10.7324/JABB.2016.40302

COCHRANE, J. C.; STROBEL, S. A. Riboswitch effectors as protein enzyme cofactors. **RNA,** v. 14, n. 6, p. 993-1002, abr. 2008. https://doi.org/10.1261/rna.908408

ESWARI, J. S.; ANAND, M.; VENKATESWARLU, C. Optimum culture medium composition for lipopeptide production by *Bacillus subtilis* using response surface model-based ant colony optimization. **Sadhana,** v. 41, n. 1, p. 55-65, jan. 2016. https://doi.org/10.1007/s12046-015-0451-x

FERNANDES, I. D. A. A.; PEDRO, A. C.; RIBEIRO, V. R.; BORTOLINI, D. G.; OZAKI, M. S. C.; MACIEL, G. M.; HAMINIUK, C. W. I. Bacterial cellulose: From production optimization to new applications. **International Journal of Biological Macromolecules,** v. 164, p. 2598-2611, dez. 2020. https://doi.org/10.1016/j.ijbiomac.2020.07.255

GEA, S.; REYNOLDS, C. T.; ROOHPOUR, N.; WIRJOSENTONO, B.; SOYKEABKAEW, N.; BILOTTI, E.; PEIJS, T. Investigation into the structural, morphological, mechanical and thermal behaviour of bacterial cellulose after a two-step purification process. **Bioresource Technology,** v. 102, n. 19, p. 9105-9110, out. 2011. https://doi.org/10.1016/j.biortech.2011.04.077

GOMES, R. J.; FARIA-TISCHER, P. C. S.; TISCHER, C. A., CONSTANTINO, L. V.; ROSA, M. F.; CHIDEROLI, R. T.; PEREIRA, U. P.; SPINOSA, W. A. *Komagataeibacter intermedius* V-05: an acetic acid bacterium isolated from vinegar industry, with high capacity for bacterial cellulose production in soybean molasses medium. **Food Technology and Biotechnology,** v. 59, n. 4, out./dez. 2021. https://doi.org/10.17113/ftb.59.04.21.7148

HUANG, H.-C.; CHEN, L.-C.; LIN, S.-B.; CHEN, H.-H. Nano-biomaterials application: *In situ* modification of bacterial cellulose structure by adding HPMC during

fermentation. **Carbohydrate Polymers,** v. 83, n. 2, p. 979-987, jan. 2011. https://doi.org/10.1016/j.carbpol.2010.09.011

LU, Z.; ZHANG, Y.; CHI, Y.; XU, N.; YAO, W.; SUN, B. Effects of alcohols on bacterial cellulose production by *Acetobacter xylinum* 186. **World Journal of Microbiology and Biotechnology,** v. 27, n. 10, p. 2281-2285, out. 2011. https://doi.org/10.1007/s11274-011-0692-8

MOHITE, B. V.; PATIL, S. V. Physical, structural, mechanical and thermal characterization of bacterial cellulose by *G. hansenii* NCIM 2529. **Carbohydrate Polymers,** v. 106, p. 132-141, jun. 2014. https://doi.org/10.1016/j.carbpol.2014.02.012

PRAJAPATI, V. S.; TRIVEDI, U. B.; PATEL, K. C. Optimization of glucoamylase production by *Colletotrichum* sp. KCP1 using statistical methodology. **Food Science and Biotechnology,** v. 22, n. 1, p. 31-38, fev. 2013. https://doi.org/10.1007/s10068-013-0005-0

SPEROTTO, G.; STASIAK, L. G.; GODOI, J. P. M. G.; GABIATTI, N. C.; SOUZA, S. S. A review of culture media for bacterial cellulose production: complex, chemically defined and minimal media modulations. **Cellulose,** v. 28, p. 2649-2673, fev. 2021. https://doi.org/10.1007/s10570-021-03754-5

TYAGI, N.; SURESH, S. Production of cellulose from sugarcane molasses using *Gluconacetobacter intermedius* SNT-1: Optimization & characterization. **Journal of Cleaner Production,** v. 112, p. 71-80, jan. 2016. https://doi.org/10.1016/j.jclepro.2015.07.054

VAIDYA, B. K.; MUTALIK, S. R.; JOSHI, R. M.; NENE, S. N.; KULKARNI, B. D. Enhanced production of amidase from *Rhodococcus erythropolis* MTCC 1526 by medium optimisation using a statistical experimental design. **Journal of Industrial Microbiology & Biotechnology,** v. 36, n. 5, p. 671-678, maio 2009. https://doi.org/10.1007/s10295-009-0536-9

YANG, Y.; JIA, J.; XING, J.; CHEN, J.; LU, S. Isolation and characteristics analysis of a novel high bacterial cellulose producing strain *Gluconacetobacter intermedius* CIs26. **Carbohydrate Polymers,** v. 92, n. 2, p. 2012-2017, fev. 2013. https://doi.org/10.1016/j.carbpol.2012.11.065

4. CAPÍTULOS

Esta Tese está organizada com apresentação dos seguintes itens (Capítulos), seguidos de Conclusão Geral e Anexos:

4.1. **Capítulo I –** REVISÃO BIBLIOGRÁFICA – Requerimentos Nutricionais de Microrganismos; Otimização de Processos Fermentativos; Bactérias do Ácido Acético (BAA); Biocelulose.

4.2. **Capítulo II –** ARTIGO CIENTÍFICO – Micronutrients iron, zinc, manganese, pantothenate and nicotinic acid play an important role in bacterial cellulose production by acetic acid bacteria.

4.3. **Capítulo III –** ARTIGO CIENTÍFICO – Effects of amino acids supplementation on bacterial cellulose production by an acetic acid bacterium *Komagataeibacter intermedius*.

4.4. **Capítulo IV –** ARTIGO CIENTÍFICO – Long pre-cultivation time before bacterial cellulose production improves the capacity of biosynthesis by a low-producing strain *Komagataeibacter hansenii*.

4.1. CAPÍTULO I – Revisão Bibliográfica

4.1.1. Requerimentos Nutricionais de Microrganismos

O cultivo de microrganismos em laboratórios ou industrialmente é realizado por inoculação de células viáveis em meios de cultura contendo nutrientes que têm a finalidade de propiciar o desenvolvimento microbiano e favorecer a formação dos produtos desejados (SOUZA; VOLPATO; HECK, 2017; SPEROTTO et al., 2021). O crescimento celular e o acúmulo de produtos metabólicos são influenciados por parâmetros físicos (pH e temperatura) e pela composição nutricional do meio de cultivo (fontes de carbono, nitrogênio, íons inorgânicos e fatores de crescimento) (JAWAN et al., 2020). As necessidades nutricionais de microrganismos variam conforme a linhagem e os diferentes microrganismos podem ter exigências nutricionais distintas. Portanto, o conhecimento das necessidades nutricionais de cada microrganismo é necessário para fornecer os nutrientes certos e em proporção adequada. Igualmente, ao conduzir um processo de fermentação é importante utilizar um meio de cultivo apropriado, pois a composição do meio pode influenciar significativamente no processo, bem como na concentração, no rendimento e na produtividade do produto desejado (KENNEDY; KROUSE, 1999; LO; LEE; LIU, 2020; MADIGAN et al., 2016; SINGH et al., 2017).

Para se formular um meio de produção ideal, há necessidade de selecionar nutrientes certos e em níveis adequados, pois isto pode fornecer um microambiente mais favorável capaz de propiciar um crescimento microbiano mínimo e necessário para se obter a máxima produção de metabólitos desejáveis. A nutrição microbiana desempenha um papel importante no início e na intensidade do metabolismo, não só porque pode restringir o crescimento celular, mas também porque a escolha adequada dos nutrientes pode resultar em efeitos metabólicos distintos. Desta forma, as pequenas alterações na composição do meio de cultivo exercem enorme impacto sobre o perfil metabólico geral dos microrganismos e na quantidade e qualidade dos metabólitos produzidos (MOBEEN; SANKAR, 2018; NURFARAHIN; MOHAMED; PHANG, 2018; SINGH et al., 2017).

Os meios de cultura utilizados para cultivo de microrganismos e obtenção de metabólitos podem ser classificados como sintéticos/definidos ou complexos. Os

meios de cultura sintéticos são preparados pela adição precisa de compostos orgânicos e inorgânicos. Portanto, é conhecida a sua composição química exata. Nos meios de cultura complexos, a composição química não é precisamente conhecida, pois apresentam componentes altamente nutritivos, tais como: extrato de levedura, soro de leite, extrato de carne, entre outros (SPEROTTO et al., 2021).

4.1.1.1. Macro e microelementos

Os microrganismos são seres vivos microscópicos que, assim como outros organismos vivos, necessitam de fontes de elementos químicos que são essenciais para o seu crescimento. Na formulação de um meio de produção, a primeira etapa é o equilíbrio estequiométrico baseado no crescimento celular e na geração de produto conforme segue (NURFARAHIN; MOHAMED; PHANG, 2018):

Fonte de Carbono e Energia + Fonte de Nitrogênio + Calor + Nutrientes → Dióxido de Carbono + Água + Biomassa + Produtos

Portanto, os processos biotecnológicos podem ser considerados como uma transformação de nutrientes e outros componentes em biomassa e produtos metabólicos (MANDENIUS; BRUNDIN, 2008).

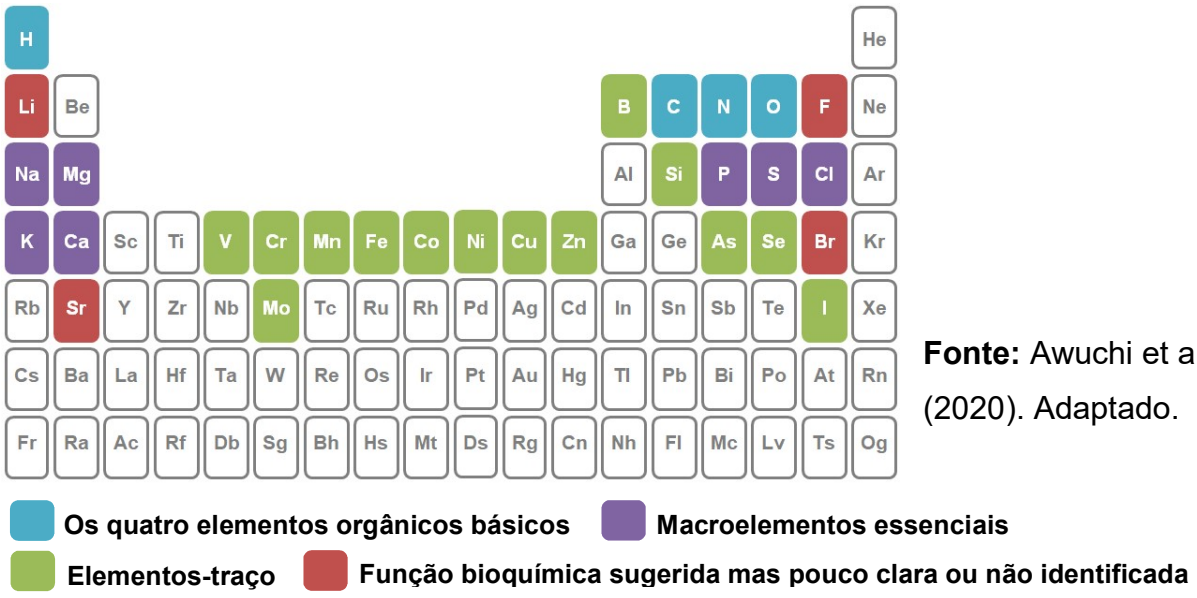
Os macronutrientes são aqueles elementos requeridos em grandes quantidades enquanto os micronutrientes são necessários apenas em quantidades traço. Entre os macronutrientes requeridos por microrganismos destacam-se o carbono, nitrogênio e fósforo. Esses elementos compõem as diversas moléculas importantes como os carboidratos, lipídeos, proteínas e ácidos nucleicos. As fontes de carbono e nitrogênio desempenham um papel importante na fermentação e estão diretamente ligadas à formação do produto e produtividade, uma vez que a biomassa microbiana e os produtos metabólicos são compostos principalmente por esses elementos. Uma célula bacteriana, por exemplo, é composta, em peso seco, por 50% de carbono e 13% de nitrogênio (GRAHOVAC et al., 2014; LUTHRA et al., 2015; MADIGAN et al., 2016).

O carbono é o componente mais importante do meio de cultivo para os microrganismos, pois é uma fonte de energia fundamental para desenvolvimento e crescimento. O carbono desempenha um papel importante no crescimento celular,

na síntese de material celular e na produção de metabólitos primário e secundário. A glicose é um exemplo típico de fonte de carbono que é metabolizada facilmente por microrganismos pela via glicólise para geração de energia. O nitrogênio é outro nutriente essencial e também necessário para o crescimento microbiano e produção de certos metabólitos primários e secundários. Ele está presente em proteínas, ácidos nucleicos e outros compostos celulares.

Os dois tipos de fontes de nitrogênio que podem ser utilizadas pelos microrganismos são os de fontes orgânicas e os de fontes inorgânicas. O nitrogênio orgânico, presente em aminoácidos e em extratos de levedura, extratos de carne e peptona, contém o carbono e tem sido considerado auxiliar significativamente no crescimento celular e na formação de polissacarídeos em comparação com o nitrogênio inorgânico. O nitrogênio inorgânico é composto principalmente pela combinação de íons positivos e negativos, tais como os compostos NH_4NO_3 e $(NH_4)_2SO_4$. O uso de aminoácidos específicos em meios de cultivo pode aumentar a produtividade da fermentação e em alguns casos pode ocorrer o inverso, quando aminoácidos inadequados inibem a síntese de metabólitos. Em processos fermentativos nos quais os precursores de produtos específicos não são adicionados ao meio de cultivo, as fontes de carbono e nitrogênio presentes podem iniciar a biossíntese dos precursores que regulam o metabolismo e influenciar a síntese do produto final (NURFARAHIN; MOHAMED; PHANG, 2018; SINGH et al., 2017; YIM; SONG; KIM, 2017).

Figura 1. Tabela periódica dos elementos essenciais.



Fonte: Awuchi et al. (2020). Adaptado.

Há muitos outros elementos que podem ser requeridos pelos microrganismos (Figura 1). O fósforo, por exemplo, é um elemento básico e necessário para a produção de fosfolipídios presentes nas membranas das células microbianas e para a produção de ácidos nucléicos, sendo que geralmente é fornecido à célula na forma de íon fosfato (PO_4^{2-}). Os elementos-traço são aqueles que são necessários em quantidades mínimas para sobrevivência dos organismos. Estes íons metálicos são importantes para o metabolismo celular porque atuam principalmente como componentes estruturais ou cofatores de enzimas. No entanto, podem se tornar tóxicos quando presentes em excesso (KOUR et al., 2019; NURFARAHIN; MOHAMED; PHANG, 2018; PRASHANTH et al., 2015; SINGH et al., 2017).

4.1.1.2. Vitaminas

As vitaminas compreendem um grupo de compostos orgânicos presentes naturalmente nos alimentos em baixas quantidades e são consideradas, do ponto de vista nutricional, como micronutrientes essenciais para a função normal dos organismos (FENNEMA; DAMODARAN; PARKIN, 2017). De acordo com as solubilidades (Quadro 1), as vitaminas são classificadas em vitaminas lipossolúveis (vitaminas A, D, E e K) e vitaminas hidrossolúveis, (vitaminas C e as do complexo B) (AWUCHI et al., 2020; PANAGOPOULOU; CHIOU; KARATHANOS, 2019).

Ao contrário de outras classes de nutrientes, as vitaminas não desempenham funções estruturais e não fornecem energia significativa no seu catabolismo. Entretanto, cada vitamina tem uma função altamente específica no organismo, sendo que são necessárias apenas em quantidades mínimas para o funcionamento adequado das funções fisiológicas normais e do metabolismo. Por exemplo, as vitaminas podem atuar como cofatores enzimáticos ou como seus precursores (vitaminas A, C, K e complexo B). Ainda, podem atuar como componentes do sistema de defesa antioxidante (vitaminas C e E) e como cofatores nas reações metabólicas de oxidação-redução (vitaminas C, E, K, niacina, riboflavina e ácido pantotênico). Embora as vitaminas compartilhem essas características gerais, apresentam poucas semelhanças químicas conforme está apresentado nas Figuras A1 e A2. Portanto, a classificação como vitaminas é estritamente empírica na qual se consideram o seu desempenho e função (COMBS JR.; MCCLUNG, 2016; COSTA-PINTO; GANTNER, 2020; ZEČIĆ; DHONDT; BRAECKMAN, 2019).

Quadro 1. Nomenclatura e classificação das principais vitaminas.

Vitamina	Principal composto	Solubilidade
A	Retinol	Lipossolúvel
B1	Tiamina	Hidrossolúvel
B2	Riboflavina	Hidrossolúvel
B3	Niacina/Ácido nicotínico	Hidrossolúvel
B5	Ácido pantotênico	Hidrossolúvel
B6	Pirodoxina	Hidrossolúvel
B7	Biotina	Hidrossolúvel
B9	Ácido fólico	Hidrossolúvel
B12	Cobalamina	Hidrossolúvel
C	Ácido ascórbico	Hidrossolúvel
D2	Ergocalciferol	Lipossolúvel
D3	Colecalciferol	Lipossolúvel
E	Tocoferol	Lipossolúvel
K1	Filoquinona	Lipossolúvel
K2	Menaquinona	Lipossolúvel

Fonte: Fennema; Damodaran; Parkin (2017). Adaptado.

4.1.1.3. Aminoácidos

Além das vitaminas, outros compostos de natureza orgânica podem ser fatores de crescimento para um microrganismo, tais como os aminoácidos, purinas e pirimidinas, nucleotídeos e nucleosídeos (BONNET et al., 2020; WALKER; STEWART, 2016). Os aminoácidos são as unidades estruturais básicas das proteínas. São constituídos de um átomo de carbono covalentemente ligado a um átomo de hidrogênio, um grupo amino ($-NH_2$), um grupo carboxila (-COOH) e um grupo comumente referido como cadeia lateral (CAMPBELL, 2017).

Na natureza há mais de 700 aminoácidos, no entanto, apenas 20 são utilizados em células vivas para a síntese de proteínas e, portanto, são considerados como fundamentais para todas as formas de vida (WU et al., 2014). As estruturas químicas destes compostos (Tabela A1) diferem apenas nas suas cadeias laterais e são estas que determinam todas as suas propriedades físico-químicas, tais como, a

carga líquida e solubilidade em água (FENNEMA; DAMODARAN; PARKIN, 2017; TRIPATHY et al., 2018). Como nas proteínas quase todos os grupamentos carboxila e amino são combinados para formar as ligações peptídicas e não estão disponíveis para interações moleculares, também é a natureza das cadeias laterais que determina a conformação de uma proteína e o tipo de interação que um aminoácido pode desempenhar nesta (ZHOU et al., 2019).

Os aminoácidos podem ser classificados em três grupos com base nas interações intra e intermoleculares que as cadeias laterais podem fornecer às proteínas: são os aminoácidos apolares (glicina, alanina, valina, leucina, isoleucina, prolina, fenilalanina, triptofano e metionina), aminoácidos polares (serina, treonina, cisteína, tirosina, asparagina e glutamina), e aminoácidos com carga positiva ou negativa (ácido aspártico, ácido glutâmico, histidina, lisina e arginina). Ainda, os aminoácidos podem ser agrupados com base nos grupos funcionais presentes na cadeia lateral, tais como: aminoácidos sulfurados (cisteína e metionina), aminoácidos aromáticos (fenilalanina, triptofano e tirosina), aminoácidos contendo grupamento ácido (ácido aspártico e ácido glutâmico), aminoácidos contendo grupamento básico (lisina, histidina e arginina) e aminoácidos contendo grupamento amina (asparagina e glutamina) (AZAD, 2018; BANY SAYD, 2014).

As proteínas e peptídeos constituídos por aminoácidos são biomoléculas vitais para a regulação de processos bioquímicos em organismos vivos, incluindo as enzimas que catalisam muitas reações bioquímicas (AKRAM et al., 2011; YOGANATHAN; VEDERAS, 2010).

4.1.2. Otimização de Processos Fermentativos

Em processos que envolvem a fermentação, há múltiplos parâmetros que podem ser simultaneamente variados. A otimização de meios de cultura e condições do processo, geralmente é realizada utilizando as metodologias estatísticas ou tradicionais que visam o aumento da produtividade. O método clássico “one-factor-at-a-time” utiliza a lógica de variar a concentração de um componente por vez, mantendo constantes as condições dos demais fatores. O nível do fator a ser investigado é então alterado em um intervalo desejado para avaliar os seus efeitos em uma resposta. Após obter o melhor valor, esta variável é fixada e as outras são alteradas até que todas tenham sido consideradas.

A prática convencional de otimização de um fator por vez apresenta a vantagem de ser simples e fácil, porém há algumas limitações. Por exemplo, não representa o efeito combinado de todos os fatores envolvidos e não leva em consideração as interações entre os diferentes fatores, especialmente quando essas interações são significativas (DINARVAND; REZAEE; FOROUGHI, 2017; RODRIGUES; IEMMA, 2014; WANG; WAN, 2009). Desta forma, o ponto ótimo do processo pode não ser corretamente identificado. Além disso, este método requer um grande número de experimentos, especialmente quando há elevado número de fatores (LOUHICHI et al., 2019; FUKUDA et al., 2018; SINGH; SHARMA; GUPTA, 2017; WANG; WAN, 2009). Outra metodologia tradicional utiliza uma matriz onde são investigadas todas as combinações dos diferentes fatores para obter no final a resolução do problema. A vantagem deste método é que explora todo o espaço experimental, porém, a desvantagem é que exige um grande número de experimentos (RODRIGUES; IEMMA, 2014).

Os experimentos estatísticos que utilizam esquemas fatoriais são mais adequados para determinar as possíveis interações entre as variáveis investigadas e selecionar a melhor condição destas variáveis para uma resposta desejada. Neste planejamento experimental, inclui-se as combinações entre os diferentes níveis de variáveis independentes, o que permite avaliar as interações entre estas variáveis (FUKUDA et al., 2018; WANG; WAN, 2009).

Os métodos estatísticos visam estimar a previsão da resposta a valores ainda não testados. Eles são econômicos pois requerem menor número de experimentos mesmo quando há um grande número de fatores. Para planejar um conjunto de experimentos de um bioprocesso visando avaliar ou otimizar as variáveis relevantes, o delineamento fatorial tem sido utilizado como uma metodologia importante pois evita erros experimentais e reduz o número de experimentos. A metodologia na qual utiliza o delineamento fatorial associado à análise de superfície de resposta é uma ferramenta com base em teorias estatísticas que fornece informações mais confiáveis sobre um determinado processo (FUKUDA et al., 2018; JACYNA; KORDALEWSKA; MARKUSZEWSKI, 2019; RODRIGUES; IEMMA, 2014).

A metodologia de superfície de resposta (MSR) se refere a uma coleção de técnicas experimentais, métodos matemáticos e inferência estatística que são utilizados para construir e explorar uma relação funcional entre as variáveis dependentes e suas interações com a função-resposta. As estratégias da aplicação

da MSR são eficazes para investigar processos complexos com objetivo de otimizar uma resposta de interesse influenciada por diversas variáveis importantes (KOECH; MUTISO; KOSKEI, 2017; BEHERA et al., 2018; HAMMAMI et al., 2020). O modelo matemático previsto pelo uso da MSR descreve com precisão o processo geral e fornece uma resposta em função das variáveis relevantes determinadas para o processo. A otimização do processo é realizada a partir da análise dos modelos matemáticos com construção de gráficos de superfícies de resposta, no qual se avalia os efeitos dos fatores e seleciona-se as condições ótimas dos fatores para as respostas investigadas (BERINGHS et al., 2015; MEDEIROS et al., 2020; MOURABET et al., 2017; YOLMEH; JAFARI, 2017).

A otimização utilizando o planejamento fatorial e análise de superfície de resposta tem sido uma prática comum na produção de biomoléculas e tem sido aplicado com frequência para otimização da concentração de constituintes do meio de cultura, além de outras variáveis críticas, tais como, temperatura, pH, aeração, entre outros (AJE; ADIE, 2020). Assim sendo, a otimização das condições nutricionais e ambientais tem desempenhado um papel importante no desenvolvimento de processos fermentativos e na melhoria do seu desempenho (JO et al., 2008). Nesses processos, projetar um meio de produção adequado e eficiente tem sido um pré-requisito para obter alto rendimento do produto e com menor custo (SHARMILA et al., 2013; WANG et al., 2008).

Na aplicação do delineamento fatorial, o primeiro passo é determinar quais fatores são os mais relevantes entre um grande número de variáveis que interferem na resposta investigada. Em algumas situações, pode haver um grande número de fatores que influenciam no processo, porém, não significa que todos os fatores apresentam efeitos significativos sobre ele. Em geral, os fatores que influenciam o processo recebem maior atenção do que aqueles que não têm grande influência, uma vez que os primeiros são mais essenciais que os últimos. Assim, a triagem inicial dos fatores pode ser realizada através da análise da significância dos efeitos destas variáveis para entender o seu papel na formação do produto e, então, os componentes significativos podem ser selecionados para uma posterior otimização (EJAZ; AHMED; SOHAIL, 2018; WANG; WAN, 2009).

Em experimentos fatoriais completos, o número de fatores aumenta exponencialmente levando a um número incontrolável de experimentos. Ao executar um planejamento fatorial incompleto, um resultado confiável pode ser obtido com

apenas alguns experimentos, após o qual pode-se determinar a direção mais favorável para se mover e encontrar um valor ou região ótima para o processo em análise. Assim, este primeiro passo de identificação de fatores que apresentam efeitos significativos é denominado de planejamento fatorial fracionado, uma vez que apenas uma fração das possíveis combinações é investigada (MANDENIUS; BRUNDIN, 2008; JU et al., 2018).

O delineamento de Plackett & Burman (PLACKETT; BURMAN, 1946) é um planejamento fatorial fracionado de dois níveis, extensivamente usado para selecionar fatores importantes em processos fermentativos que envolvem grande número de variáveis. Com base na análise de variância (ANOVA) do modelo estimado, os fatores significativos podem ser identificados e selecionados para investigações posteriores (GÜNDOĞDU et al., 2016; WARDA et al., 2016). Esse delineamento fatorial tem sido ideal para identificar as variáveis mais importantes, quando há muitos fatores a serem analisados, há pouco conhecimento do processo e ainda, se está longe das condições desejadas ou otimizadas (RODRIGUES; IEMMA, 2014). O planejamento fatorial de Plackett & Burman permite estimar a variabilidade do erro aleatório e testar a significância estatística dos parâmetros com um número menor de experimentos (FERREIRA et al., 2017; YIN et al., 2017). Nesse método não se determina a quantidade exata e também não se consideram as interações entre as variáveis. No entanto, pode-se obter informações importantes sobre cada fator, sendo muito útil em estudos preliminares nos quais o objetivo principal é selecionar as variáveis que podem ser corrigidas ou eliminadas em processos de otimização posteriores (HOSNY et al., 2018; SINGH et al., 2013; VERMA et al., 2017).

Após a triagem inicial, o passo seguinte é otimizar as variáveis selecionadas. Basicamente, este processo de otimização envolve quatro etapas principais: (i) realizar os experimentos estatisticamente planejados; (ii) estimar os coeficientes em um modelo matemático; (iii) prever sua resposta e, finalmente, após a construção e otimização do modelo; (iv) verificar a sua adequação (ESKANDARI et al., 2020; BEHERA et al., 2018). O modelo desenvolvido e estimado é utilizado para construção de gráficos de contorno que fornecem a variação das respostas quando os níveis de duas das variáveis são alterados. Estes gráficos são utilizados para determinar as condições ótimas das variáveis e suas interações para a resposta desejada. Um gráfico de contorno circular indica que as interações entre as variáveis

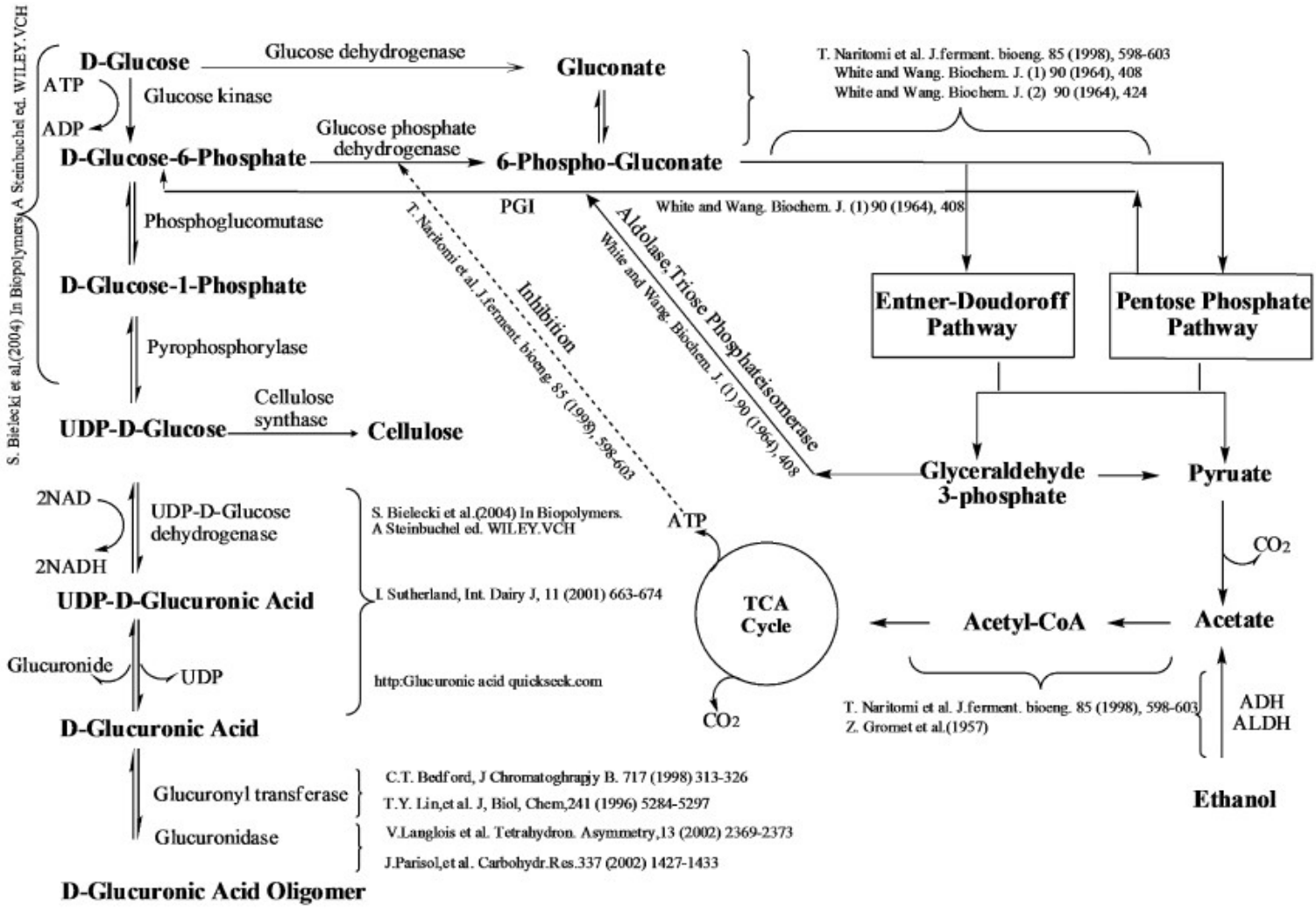
correspondentes são desprezíveis, enquanto que um gráfico de contorno elíptico indica o contrário (MOHAMMADI et al., 2016; REGTI et al., 2017; TAYEB; TONY; MANSOUR, 2018). A aplicação de técnicas estatísticas com planejamentos experimentais em processos industriais tem sido utilizada, pois pode resultar em melhor rendimento do produto, na redução na variabilidade do processo, na confirmação mais próxima da resposta e na redução do tempo e custos gerais do processo (BOUDRAHEM; ZIANI; AISSANI-BENISSAD, 2019).

4.1.3. Bactérias do Ácido Acético

As bactérias do ácido acético (BAA) são bactérias Gram-negativas ou Gram-variáveis, estritamente aeróbias que estão presentes principalmente em frutas e vegetais e em processo de decomposição. As espécies de BAA são bem conhecidas por terem uma alta capacidade de metabolizar álcoois, açúcares ou álcoois de açúcar, como por exemplo, etanol, glicose, glicerol, sacarose, frutose, manitol e outros, na presença de oxigênio, a temperaturas entre 25 e 30 ºC e pH entre 3 e 7. Como resultado destas atividades oxidativas com produção de energia, ocorre um acumulo de produtos de oxidação no meio de cultura, como os ácidos carboxílicos, aldeídos e cetonas (CACICEDO et al., 2016; DE ROOS; DE VUYST, 2018; QIU; ZHANG; HONG, 2021; LYNCH et al., 2019). Essas reações oxidativas são catalisadas por desidrogenases ligadas à membrana, localizadas no espaço periplasmático da membrana celular (CHINA et al., 2018; GOMES et al., 2018).

As principais vias metabólicas em BAA são: (i) a via respiratória de oxidação do etanol; (ii) o ciclo do ácido tricarboxílico; (iii) a via da hexose monofosfato; e (iv) a via da pentose fosfato. Elas catabolizam os açúcares por meio da via citoplasmática da hexose monofosfato. A glicólise está ausente devido à ausência de fosfofrutoquinase. A via Entner-Doudoroff ocorre apenas em linhagens que sintetizam celulose, onde parece ser mais ativa que o ciclo da hexose monofosfato. A capacidade de crescer em glicerol é devida à gliconeogênese (QIU; ZHANG; HONG, 2021; SIEVERS; SWINGS, 2015). Segundo Huang et al. (2014), em *Komagataeibacter xylinus*, as duas vias metabólicas principais que estão presentes são o ciclo da pentose fosfato para a oxidação de carboidratos e o ciclo do ácido tricarboxílico para a oxidação de ácidos orgânicos (Figura 2).

Figura 2. Vias metabólicas propostas para a produção de biocelulose.



Fonte: Ha et al. (2011).

As BAA pertencem à família Acetobacteraceae e são os principais microrganismos envolvidos na produção de vinagre devido à sua elevada capacidade de produzir ácido acético a partir do etanol na presença de oxigênio (VALERA et al., 2015; QIU; ZHANG; HONG, 2021). O perfil aeróbio destas bactérias impõe a presença de oxigenação suficiente para proporcionar crescimento e formação de produtos (YASSINE et al., 2016).

Os gêneros de BAA apresentam uma diferença na capacidade de oxidação de etanol, açúcar e álcool de açúcar. Por exemplo, em *Acetobacter*, a capacidade para oxidar o etanol excede ou ao menos permanece no mesmo nível do que a capacidade de oxidar glicose. Contrariamente, um elevado acúmulo de ácido

glucônico, que excede aquele de ácido acético, é observado nas espécies do gênero *Gluconobacter*. Esse fato demonstra que o gênero *Gluconobacter*, além de exibir forte atividade cetogênica sobre poliálcoois como manitol, sorbitol e glicerol, oxida principalmente a glicose para o ácido glucônico. Por outro lado, as espécies do gênero *Acetobacter* geralmente não exibem a mesma capacidade oxidativa sobre açúcares e poliálcoois, oxidando principalmente o etanol para o ácido acético (GOMES et al., 2018).

Entre os dezenove gêneros atualmente descritos dentro da família Acetobacteraceae, os gêneros *Acetobacter*, *Gluconobacter*, *Gluconacetobacter* e *Komagataeibacter* são os principais responsáveis pela produção de vinagre devido à sua alta capacidade em oxidar o etanol a ácido acético bem como à alta resistência destes gêneros ao ácido acético liberado para o meio fermentativo (XIA et al., 2021; ANDRÉS-BARRAO et al., 2013; GOMES et al., 2018). Muitas outras espécies de bactérias são também capazes de oxidar o etanol sob condições aeróbicas, mas são incapazes de oxidá-lo sob condições de elevada acidez (GOMES et al., 2018).

As BAA oxidam o etanol para formação do ácido acético por meio de duas reações sequenciais. Primeiro, a enzima álcool desidrogenase (ADH) ligada à membrana, catalisa a oxidação do etanol para acetaldeído. Sequencialmente, o acetaldeído gerado é imediatamente oxidado a ácido acético, catalisado pela enzima aldeído desidrogenase (ALDH) ligada à membrana e localizada próximo à ADH (CHINA et al., 2018; GOMES et al., 2018; QIU; ZHANG; HONG, 2021).

Durante a oxidação do álcool, nenhuma liberação de aldeído é observada, indicando que ADH e ALDH formam um complexo multienzimático na membrana bacteriana, ambas funcionando sequencialmente para produzir o ácido acético a partir de etanol (GOMES et al., 2018). O ácido produzido é liberado para meio de crescimento onde se acumula a um máximo de 5-8% (m/v) nas espécies de *Acetobacter* e de 10-20% (m/v) nas espécies de *Komagataeibacter*. Os diferentes valores de concentração estão relacionados a diferenças na capacidade de resistência das espécies destes gêneros ao ácido acético liberado, o qual é altamente tóxico a microrganismos em geral. Tem-se descrito que a perda na atividade da enzima ADH reduz a resistência ao ácido acético (ANDRÉS-BARRAO et al., 2016; QIU; ZHANG; HONG, 2021; WU et al., 2017). Alguns gêneros podem oxidar ainda mais o ácido acético para CO_2 e H_2O, resultando na oxidação de acetato ou superoxidação. Esta habilidade é útil para a distinção do gênero

Gluconobacter, que não apresenta a mesma capacidade. Esta condição depende da composição do meio, ocorrendo principalmente quando o etanol foi totalmente consumido (QIU; ZHANG; HONG, 2021; GOMES et al., 2018).

O vinagre, principal produto obtido pelas BAA, é uma solução aquosa de ácido acético, conhecido e consumido no mundo todo como condimento alimentar e conservante. Ele retarda o crescimento microbiano e contribui com propriedades sensoriais aos alimentos em que é utilizado (ADEBAYO-OYETORO et al., 2017; SPINOSA et al., 2015). Este produto é geralmente obtido pela fermentação de fontes de carboidratos, como glicose ou amido hidrolisado, num processo dividido em duas etapas: (i) primeiro uma fermentação anaeróbica, conduzida por leveduras do gênero *Saccharomyces cerevisiae* que produzem etanol (Figura 3 - Reação 1) e (ii) seguido por uma oxidação do etanol para formação do ácido acético conduzida pelas BAA (Figura 3 - Reação 2). As matérias-primas constituídas de amido ou carboidratos complexos necessitam ainda de sacarificação anterior à fermentação alcoólica, para liberação de açúcares fermentescíveis (GOMES et al., 2018; HO et al., 2017).

Além da sua importância na oxidação do etanol para produção de vinagre, algumas espécies de BAA são também descritas como capazes de sintetizar outras moléculas importantes, como sorbose a partir de sorbitol, dihidroxiacetona a partir de glicerol e exopolissacarídeos tais como biocelulose e levana (CHINA et al., 2018; ZHANG et al., 2017).

Figura 3. Reações químicas ocorridas nas etapas de fermentação alcoólica e acética para obtenção do vinagre.

1ª Reação (Fermentação alcóolica):

$$C_6H_{12}O_6\ (aq) \xrightarrow[\text{anaerobiose}]{\text{levedura}} 2\ CH_3CH_2OH\ (aq) + 2\ CO_2\ (g)$$

2ª Reação (Oxidação):

$$CH_3CH_2OH\ (aq) + O_2\ (g) \xrightarrow[\text{ar}]{\text{bactéria acética}} CH_3COOH\ (aq) + H_2O\ (l)$$

Em relação à nutrição, as fontes de carbono mais adequadas para o crescimento de BAA são glicose, arabinose, manitol, sorbitol e glicerol. Elas não hidrolisam o amido e nem lactose. O etanol, piruvato e lactato não são assimilados sob condições normais, mas podem ser utilizados na presença de fatores de crescimento encontrados em fontes complexas. Por exemplo, a adição de vitaminas do complexo B foi capaz de promover o crescimento de espécies incapazes de crescer com etanol como única fonte de carbono (ASAI, 1968). Em estudo sobre a assimilação de glicose e etanol como fontes de carbono por espécies de *Acetobacter* e de *Gluconobacter*, foi observado que as linhagens com predominante metabolismo de lactato, assimilando principalmente etanol, pertenciam ao gênero *Acetobacter*. Por outro lado, aquelas que apresentaram predominante metabolismo glicolítico, assimilando principalmente glicose, pertenciam exclusivamente ao gênero *Gluconobacter* (ASAI, 1968).

Em relação às fontes de nitrogênio, foi relatado que várias linhagens de BAA apresentam a habilidade de crescer em meio quimicamente definido, utilizando sais de amônio (íons NH_4^+) como única fonte de nitrogênio quando etanol ou ácido acético são utilizados como fonte de carbono. Outras espécies, diferentemente, requerem para isso fontes de nitrogênio orgânico ou a presença de fatores de crescimento como tiamina, ácido pantotênico, ácido nicotínico e ácido *p*-aminobenzóico. A assimilação de sais de amônio pelas BAA em meio sintético dependeria, assim, do tipo de fonte de carbono utilizada e, em algumas espécies, da presença de aminoácidos, vitaminas e bases nitrogenadas como fatores de crescimento. Espécies de BAA capazes de utilizar íons nitrato (NO_3^-) como fonte de nitrogênio também foram relatadas (MOHITE; KAMALJA; PATIL, 2012; ASAI, 1968).

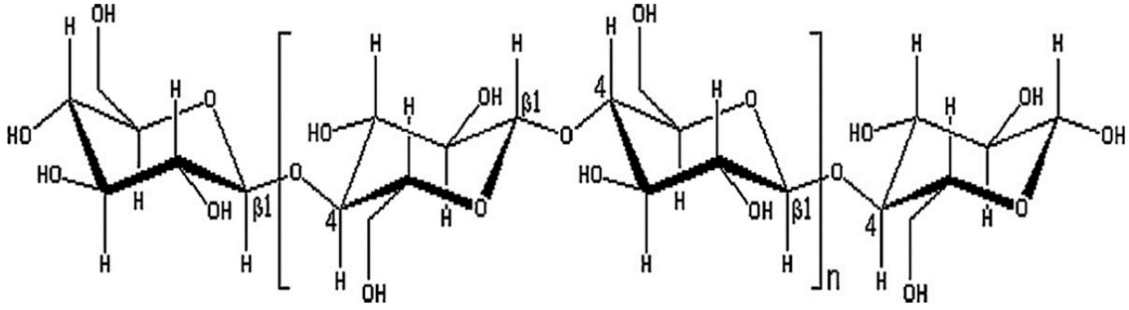
Os fatores de crescimento requeridos pelas BAA também dependem do fornecimento da fonte de carbono. Em seu estudo, Santos Junior et al. (2021) verificaram que diferentes linhagens de BAA não só requerem diferentes minerais e vitaminas como também quantidades distintas desses nutrientes quando se utiliza manitol como fonte de carbono. Por exemplo, uma linhagem de coleção de cultura *Acetobacter aceti* CCT 2565 necessitou de mais micronutrientes do que a linhagem *Acetobacter* sp., isolada de indústria de vinagre. Em outro trabalho, foi observado pelo mesmo autor que os microminerais exerceram efeitos significativos sobre a produtividade de ácido acético em processo submerso de fermentação acética. Enquanto que zinco e boro exerceram efeito negativo sobre a produtividade, por

outro lado o ferro, molibdênio e manganês exerceram efeitos positivos, sendo observado que o aumento da sua concentração no meio de fermentação aumentou a produtividade do processo (SANTOS JUNIOR, 2009).

4.1.4. Biocelulose

A celulose é um homopolímero linear, de alto peso molecular e insolúvel em água. É constituída de unidades repetidas de D-glucopiranose $(C_6H_{10}O_5)_n$ alternadamente giradas em 180º que estão unidas por ligações glicosídicas do tipo β-(1→4) (Figura 4) (GIBSON, 2012; ZHAO et al., 2019). A celulose é o biopolímero renovável mais abundante da Terra. É obtida principalmente a partir de fontes vegetais, sendo presente como o componente principal da parede celular das plantas. No entanto, algumas linhagens de bactérias também podem produzir a celulose extracelularmente (ISLAM et al., 2017; WANG; TAVAKOLI; TANG, 2019).

Figura 4. Estrutura da celulose.



Fonte: Sheykhnazari et al. (2011).

A biocelulose de origem microbiana é um exopolissacarídeo de celulose puro e produzido principalmente por espécies de BAA a partir de diferentes fontes de carbono. As espécies deste grupo que apresentam capacidade de produção de celulose, como *Gluconacetobacter* spp. e *Komagataeibacter* spp., são consideradas promissoras para a biossíntese, pois: (i) podem ser cultivadas em laboratório; (ii) são comumente encontradas em frutas e produtos derivados; (iii) não são patogênicas e (iv) produzem biocelulose com rendimento relativamente elevado quando comparado com outras espécies (BILGI et al., 2016). Este biopolímero é idêntico à celulose vegetal quanto à sua fórmula molecular e estrutura química. No entanto,

ambas apresentam diferenças significativas em relação à pureza, características e propriedades físicas (BLANCO PARTE et al., 2020; VASCONCELOS et al., 2017; WANG; TAVAKOLI; TANG, 2019).

A celulose de origem bacteriana é livre de outras moléculas, tais como, a lignina, pectina e hemicelulose, o que elimina a necessidade de utilizar um tratamento químico severo e energia para a remoção destes compostos. A biocelulose apresenta-se misturada apenas com células microbianas, nutrientes e outros metabólitos secundários, os quais podem ser removidos por tratamento alcalino suave, produzindo uma celulose de elevada pureza. Desta forma, seu processo de purificação é relativamente simples, de baixo custo e impacto ambiental mais reduzido (ANDRIANI; APRIYANA; KARINA, 2020; CAZÓN; VÁZQUEZ, 2021; LIN et al., 2020; LOTFIMAN et al., 2018; VASCONCELOS et al., 2017).

A celulose extracelular foi relatada pela primeira vez por Adrian Brown em 1886 quando o mesmo realizava a fermentação acética para obtenção do vinagre. Na ocasião, a descreveu como uma película branca, intumescida e gelatinosa, originada sobre a superfície do meio. Esta substância foi denominada de "mãe-do-vinagre" sendo posteriormente contatado que ela apresentava a mesma estrutura química, composição e reatividade da celulose vegetal (CACICEDO et al., 2016; LIN et al., 2020; SPEROTTO et al., 2021; ULLAH et al., 2016).

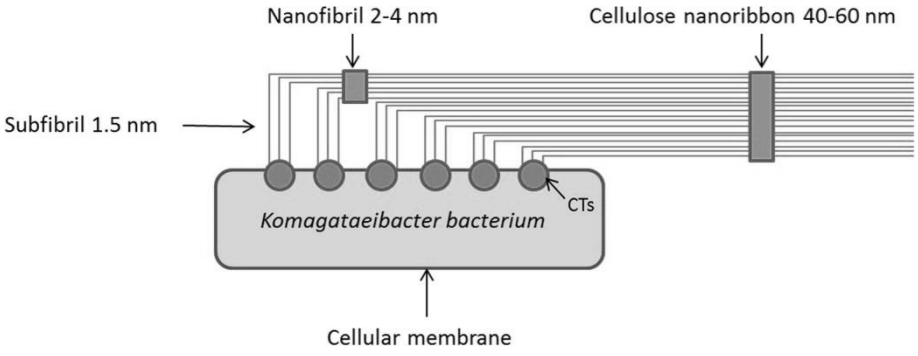
Sob condições de cultivo estático, a celulose é sintetizada na forma de um filme espesso branco e gelatinoso, consistindo de um arranjo nanofibrilar de fibras celulósicas puras, distribuídas em direções aleatórias e que aumenta em espessura com o aumento do tempo de cultivo. A celulose bacteriana é muitas vezes denominada de nanocelulose, devido às dimensões de suas fibras que são cerca de 100 vezes mais finas do que as fibras de celulose vegetal. Essas nanofibras formam uma rede tridimensional em que estão interconectadas sem qualquer orientação preferencial, como resultado de um processo biológico nos quais as cadeias de glucana são expelidas dos poros das bactérias para o meio de cultivo (FORESTI; VÁZQUEZ; BOURY, 2017; LIN et al., 2020; SEMJONOVS et al., 2017; WANG; TAVAKOLI; TANG, 2019).

Durante o processo de biossíntese, as moléculas de celulose são primeiramente sintetizadas dentro da bactéria e subsequentemente expelidas através de minúsculos poros presentes no eixo longitudinal da membrana externa da célula bacteriana. Uma única célula de *Komagataeibacter* spp. tem entre 50 e 80

poros de 3,5 nm de diâmetro para extrusão de celulose a partir de sua membrana. Através desses poros, as bactérias liberam polímeros de glicose que se unem uns aos outros em cerca de 10 a 15 cadeias poliméricas, formando uma subfibrila com um diâmetro de aproximadamente 1,5 nm (Figura 5). As subfibrilas se juntam para formar nanofibrilas (3–4 nm de espessura) e, em seguida, fitas de celulose são produzidas com uma largura aproximada de 40–80 nm e 3-8 nm de espessura. Essas fibrilas formam uma estrutura de rede tridimensional que é estabilizada por ligações de hidrogênio intra e intermoleculares por meio de grupos hidroxilas disponíveis nas moléculas (CACICEDO et al., 2016; GORGIEVA; TRČEK, 2019; CAMPANO et al., 2016; LIU et al., 2020).

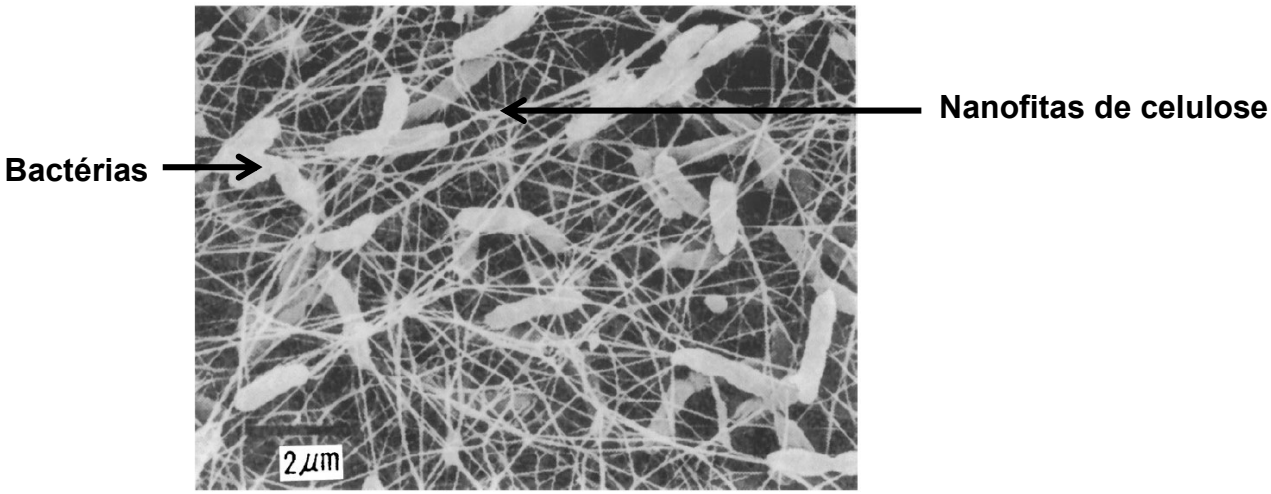
Na Figura 6 observa-se um esquema da imagem de microscopia eletrônica de varredura (MEV) das nanofitas de celulose (linhas finas brancas interpenetradas) que são excretadas a partir das células de *Komagataeibacter* spp. (bactérias brancas em forma de bastonetes). A síntese de biocelulose é resultado do metabolismo oxidativo de carboidratos e ocorre pela polimerização de monômeros de glicose em cadeias lineares de β-1,4-glucana (ESLAHI et al., 2020; OLIVEIRA et al., 2017). Essa biossíntese ocorre em cinco etapas: (1) fosforilação da glicose em glicose-6-fosfato; (2) isomerização da glicose-6-fosfato em glicose-1-fosfato; (3) conversão de glicose-1-fosfato a uridina-difosfato-glicose (UDP-glicose); (4) polimerização das cadeias de β-1,4-glucana a partir da UDP-glicose pelo complexo celulose sintase; e (5) cristalização das cadeias de β-1,4-glucana em celulose (LIU et al., 2020; LEE; PARK, 2017). Na Figura 7 observa-se o esquema da via bioquímica para biossíntese de celulose pelas bactérias.

Figura 5. Imagem esquemática da configuração das nanofitas de celulose.



Fonte: Cacicedo et al. (2016).

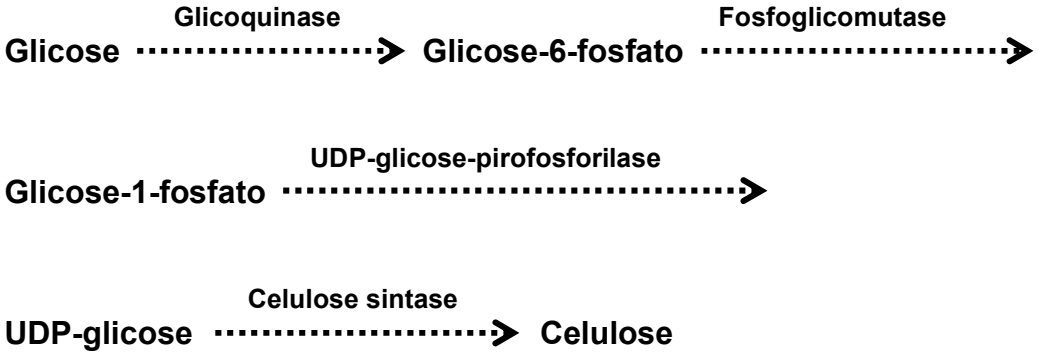
Figura 6. Micrografia eletrônica de varredura da superfície da biocelulose.



Fonte: Iguchi; Yamanaka; Budhiono (2000) apud Qiu; Netravali (2014). Adaptado.

A enzima celulose bacteriana sintase (Bcs) é um complexo proteico multicomponente codificado em um operon contendo quatro genes (BcsA, BcsB, BcsC e BcsD) dos quais os dois primeiros, bcsA e bcsB, são essenciais para a biossíntese, embora todos os quatro genes são necessários para a máxima produção de celulose (GAO et al., 2017; URBINA et al., 2017).

Figura 7. Via bioquímica para síntese de celulose em *Komagataeibacter xylinus*.



Fonte: Ahmed; Gultekinoglu; Edirisinghe (2020). Adaptado.

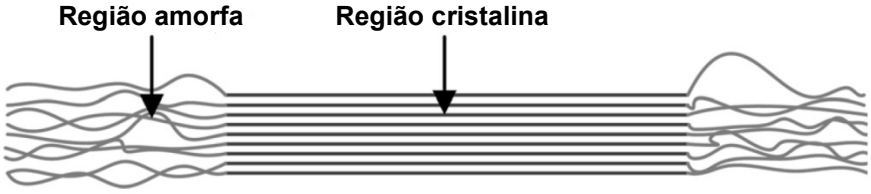
A subunidade BcsA, localizada na face citoplasmática da membrana interna possui um domínio catalítico β-1,4-glicosiltransferase responsável por polimerizar os monômeros de uridina difosfoglucose (UDP-glicose) em cadeias de celulose. O segundo gene, bcsB, codifica a subunidade reguladora da celulose sintase que se liga ao ativador alostérico diguanilmonofosfato cíclico (cdi-GMP). Esse também desempenha um papel importante como segundo mensageiro e ativa o processo de síntese de celulose. As funções de bcsC e bcsD ainda não estão totalmente esclarecidas. É possível que a extrusão de celulose do periplasma para o ambiente extracelular seja facilitada pela ação do gene bcsC, uma vez que o mesmo codifica proteínas similares às envolvidas na formação dos poros utilizados para secretar a celulose. A observação de que o bcsC é essencial para a biossíntese *in vivo*, mas não *in vitro* também sugere que este gene seja responsável pela formação de poros na membrana externa. A desativação de bcsA, bcsB e bcsC bloqueia completamente a síntese de celulose, enquanto que a desativação de bcsD diminui a produção em 40%. Isso sugere que o bcsD desempenha um papel na cristalização da celulose por facilitar a formação de ligação de hidrogênio entre as cadeias de β-glucana (AUGIMERI; STRAP, 2015; LEE et al., 2014; LIN et al., 2013).

Após a biossíntese, há formação de uma película constituída de uma rede de cadeias cristalinas de celulose alinhadas em paralelo e intercaladas entre as regiões amorfas, isto é, são regiões com arranjos menos ordenados que ocupam 90% do volume total (Figura 8) (PICHETH et al., 2017). A biocelulose é hidrofílica devido à alta densidade de grupos hidroxila em sua superfície. Por outro lado, interações hidrofóbicas ocorrem como resultado de sua organização cristalina, o que a torna anfifílica. Esta propriedade pode ser utilizada para estabilizar as emulsões sem adição de surfactante (AZEREDO et al., 2019). As nanofitas de celulose geram uma estrutura em forma de rede com muitos espaços vazios entre as fibras. Estas nanofitas bem separadas criam uma área de superfície expandida e uma matriz altamente porosa (COSTA et al., 2017).

A biocelulose se apresenta como um hidrogel reticulado. Normalmente, a película de celulose formada no meio de cultura contém entre 97 a 99% (m/m) de água e de 1 a 3% (m/m) de celulose. Esta elevada capacidade de absorção/retenção de água (até 400 vezes o seu peso seco) é principalmente devido à sua hidrofilicidade e sua estrutura de rede muito porosa que retém a água por meio de ligações de hidrogênio. Tem sido demonstrado que apenas 10% dos 99% de água

contida nos hidrogéis de celulose apresentaram comportamento de água livre, enquanto que os 89% restantes estavam ligados à rede de nanofibrilas (BLANCO PARTE et al., 2020; CAMPANO et al., 2016; CHEN et al., 2017; LIU et al., 2020). Esta característica torna a biocelulose um excelente material para cicatrização de feridas, pois ajuda a manter um meio ambiente úmido e elimina os exsudatos, garantindo uma cicatrização adequada (ESLAHI et al., 2020).

Figura 8. Esquema da nanofibrila de celulose indicando uma das configurações sugeridas para as regiões cristalina e amorfa.



Fonte: Campano et al. (2016). Adaptado.

Durante o processo de formação em condições de cultivo estático, a bactéria produz celulose apenas nas proximidades da superfície do meio de cultura devido à sua condição aeróbia. Neste método, a produção no meio de cultivo é altamente dependente da área de superfície da interface ar-líquido dos recipientes e geralmente não é afetado pelo volume e profundidade do meio (LIN et al., 2020; ANDRIANI; APRIYANA; KARINA, 2020; WANG; TAVAKOLI; TANG, 2019). A produção em cultivo estático resulta em um biofilme com superfície mais densa onde está em contato com o ar e uma rede mais gelatinosa do outro lado onde está em contato com o líquido (STUMPF et al., 2018).

Sob condições de cultivo agitado a celulose é sintetizada na forma de esferas de formato irregular. O tamanho, a forma e a quantidade de celulose produzida em meio com agitação estão relacionadas a fatores, tais como, velocidade de agitação, força de cisalhamento, fornecimento de oxigênio, tempo de cultivo e tipos de aditivos adicionados ao meio de cultura (KIM et al., 2017; ANDRIANI; APRIYANA; KARINA, 2020). A agitação do meio tem um impacto muito grande na quantidade de celulose produzida e nas suas propriedades. Enquanto a agitação resulta em aumento do

crescimento de células, ocorre uma diminuição na produção de celulose, em comparação com a produção em condições estáticas. O fato é atribuído ao aumento da aeração em sistemas agitados, o que favorece o crescimento celular e diminui a necessidade de ancoragem à película na parte superior do meio para obter maiores níveis de oxigênio. Além disso, na fermentação sob agitação, devido à instabilidade genética, algumas células podem se converter em mutantes não produtoras de celulose. A tensão de cisalhamento exercida sobre as células bacterianas causa a mutação por inativar o gene que codifica a síntese de celulose e desativa as enzimas essenciais envolvidas na síntese, o que leva à redução da capacidade de produção do biopolímero (CAMPANO et al., 2018; FORESTI; VÁZQUEZ; BOURY, 2017; AHMED; GULTEKINOGLU; EDIRISINGHE, 2020). Também, é possível que a agitação do meio diminua a produção porque as células mutantes negativas para celulose (Cel-) crescem mais rápido do que as células produtoras (Cel+), tornando o meio mais enriquecido com as células mutantes (CAMPANO et al., 2016). Em condições estáticas, as células produtoras (Cel+) migram em direção à interface ar-líquido, rica em oxigênio, onde produzem a membrana. A celulose formada limita o acesso ao oxigênio nas profundidades do meio e, assim, a maioria das células encontradas são Cel+ (AHMED; GULTEKINOGLU; EDIRISINGHE, 2020).

A agitação também tem um impacto na agregação e interação das fibrilas. Embora quimicamente idênticas, ao comparar a biocelulose produzida sob condições estáticas e agitadas, aquela obtida a partir de cultivo agitado resulta em uma perda de resistência mecânica e a um menor grau de polimerização, menor índice de cristalinidade e menor teor celulose Iα (FERNANDES et al., 2020; AHMED; GULTEKINOGLU; EDIRISINGHE, 2020).

As espécies do gênero *Komagataeibacter* produzem duas formas de celulose: a celulose I que é um polímero em forma de fita, e a celulose II que é uma forma amorfa e termodinamicamente mais estável do polímero. A celulose I é composta por cadeias de β-1,4 glucanas alinhadas em paralelo e dispostas uniaxialmente por forças de van der Waals, sendo produzida na forma de película por *K. xylinus*. Enquanto que a celulose II consiste em cadeias anti-paralelas de β-1,4 glucanas dispostas de maneira aleatória, sendo encontrada em culturas agitadas de *K. xylinus* ou após a recristalização/mercerização industrial da celulose I (MARESTONI et al., 2020; HUANG et al., 2010). Na natureza, a estrutura da celulose I é encontrada sob duas formas alomórficas: Iα (uma cadeia em uma célula unitária triclínica) ou Iβ

(duas cadeias em uma célula unitária monoclínica). A celulose pertencente às paredes celulares das plantas apresenta maior percentagem de estrutura Iβ, em comparação com a celulose originária de algas e bactérias, que apresenta maior percentagem de estrutura Iα (70-80%) (CAMPANO et al., 2016; RONGPIPI et al., 2019).

Sem nenhum aditivo, as cadeias poliméricas produzidas por bactérias acéticas são uma mistura de celulose Iα, Iβ e regiões amorfas. De forma geral, esta biocelulose se apresenta como uma rede tridiomensional de nanofibras altamente cristalinas (70 a 90%, contra 40-60% para a celulose vegetal), com alto grau de polimerização (até 8000), alto conteúdo de água (até 99%), alta moldabilidade e hidrofilicidade, e boa biocompatibilidade (FORESTI; VÁZQUEZ; BOURY, 2017; CAZÓN; VÁZQUEZ, 2021; WANG; TAVAKOLI; TANG, 2019). As propriedades da biocelulose podem ser mudadas de acordo com a linhagem do microrganismo, a composição do meio de cultura e as técnicas de cultivo utilizadas. A composição do meio de cultura impacta nas propriedades obtidas como, por exemplo, a densidade e a porosidade das membranas, que por sua vez afetam a capacidade de retenção de água. Alterando-se também as condições de cultivo, como agitação, temperatura e aditivos, é possível alterar a proporção celulose Iα / Iβ e a largura das fibrilas (DAĞBAĞLI; GÖKSUNGUR, 2017; FORESTI; VÁZQUEZ; BOURY, 2017).

O substrato utilizado para a produção é de extrema importância, e nos últimos anos, pesquisas têm sido orientadas na busca por resíduos agrícolas e industriais como fontes alternativas de nutrientes com objetivo de tornar essa produção “mais verde” e reduzir os custos de produção (FORESTI; VÁZQUEZ; BOURY, 2017). Nestes meios com alto teor de açúcar, estão incluídos os sucos de frutas (KUROSUMI et al., 2009) e os subprodutos relacionados, como cascas de frutas (GÜZEL; AKPINAR, 2019; KUMBHAR et al., 2015) e frutas em decomposição (JOZALA et al., 2015). O melaço derivado das indústrias de cana-de-açúcar (MACHADO et al., 2018), beterraba (SALARI et al., 2019) e soja (GOMES et al., 2021; SOUZA et al., 2020) também foram testados como fontes de carbono, nitrogênio e outros nutrientes para a produção microbiológica de celulose, nos quais se alcançaram bons resultados.

Para tornar a biossíntese de celulose bacteriana viável na indústria, é necessário o uso de um substrato efetivo e de baixo custo. Uma desvantagem no uso de resíduos agroindustriais para a produção é a dificuldade em padronizar o

processo, uma vez que a composição percentual do substrato pode variar de tempo em tempo e de um local para outro (QI et al., 2017; CHANDRASEKARAN; BARI; SINHA, 2017).

Outra tendência importante na produção de biocelulose é a introdução de aditivos, incluindo ácido hialurônico (TANG et al., 2021), álcool polivinílico (CHUNSHOM et al., 2018), ácido tânico (ZHANG et al., 2020), poli(ácido acrílico) (CHUAH et al., 2018), amido (SANTOS; SPINACÉ, 2021), carboximetilcelulose (FONTES et al., 2018), κ-carragena (NUMATA; YOSHIHARA; KONO, 2021), colágeno (YANG et al., 2017), gelatina (YE et al., 2019), nanopartículas metálicas (HE et al., 2018), goma xantana (GAO et al., 2020), quitosana (JIA et al., 2017), medicamentos (BADSHAH et al., 2018), hidroxiapatita (BAYIR et al., 2019), poli-hidroxibutirato (DING et al., 2021), óxido de grafeno (LUO et al., 2018), óxido de zinco (KHALID et al., 2017), pectina (CACICEDO et al., 2018), queratina (RADU et al., 2021) e xiloglucana (LU et al., 2021), foram adicionados, por exemplo, com o objetivo de produzir nanocompósitos.

Existem duas abordagens principais de modificação para produzir nanocompósitos de celulose: a modificação *in-situ*, que implica na adição de material ao meio de cultura no início do crescimento bacteriano, durante o processo de fermentação. Neste caso, a nova estrutura da celulose é o resultado do emaranhamento das nanofibrilas com as moléculas exógenas adicionadas, que se tornam parte da estrutura do polímero, produzindo matrizes com novas características e propriedades. Alternativamente, a modificação *ex-situ* é baseada na modificação da matriz de celulose após a produção e purificação, seguida pela impregnação de moléculas exógenas sobre a superfície da membrana, criando uma nova camada com diferentes propriedades físico-químicas e biológicas. Essas modificações têm várias finalidades, como a melhoria das propriedades de adesão, termoestabilidade e biocompatibilidade, aumento da atividade antimicrobiana, aumento ou diminuição da resistência mecânica, capacidade de retenção de água, entre outras propriedades específicas (CACICEDO et al., 2016; LIN et al., 2016; FORESTI; VÁZQUEZ; BOURY, 2017; KESKIN; URKMEZ; HAMES, 2017).

Na área alimentícia, a biocelulose pode ser utilizada para melhorar a reologia dos alimentos, atuando como agente espessante, estabilizante, gelificante ou agente de suspensão. Esta pode auxiliar para melhorar a textura, a viscosidade, a retenção de água, aumentar a força do gel, estabilizar emulsões, evitar a precipitação de

partículas ou ainda fornecer estabilidade ao aquecimento após tratamento térmico (PAXIMADA et al., 2016; SHI et al., 2014). Também tem sido utilizada em pesquisas para produção de alimentos com baixo teor calórico, baixo teor de gordura, como material para elaboração de embalagens biodegradáveis ou ainda para imobilização de enzimas e microrganismos probióticos (AZEREDO et al., 2019; SHI et al., 2014; ULLAH; SANTOS; KHAN, 2016).

Como ingrediente alimentar, uma das principais vantagens da biocelulose é o seu apelo em alimentos dietéticos, devido à sua indigestibilidade. Por esta razão, esta película gelatinosa pode ser uma boa fonte de fibra dietética e é geralmente consumida sob a forma de uma tradicional sobremesa originária das Filipinas, denominada de “nata-de-coco”, por ser fermentada em água de coco. No entanto, diferentes sabores podem ser preparados a partir da fermentação de diferentes sucos de frutas (AZEREDO et al., 2019; LIN et al., 2020; FORESTI; VÁZQUEZ; BOURY, 2017). Quando comparada com outras fibras alimentares, a biocelulose tem várias vantagens, tais como: (i) ser altamente pura e não requerer tratamentos químicos severos para seu isolamento e purificação; (ii) as bactérias podem utilizar xaropes de frutas para secretar celulose no meio de cultura, com o sabor e o pigmento da fruta utilizada e (iii) as fibras estão em uma escala nanométrica com uma estrutura fina de rede tridimensional, podendo ser obtida em uma variedade de formas e texturas, o que possibilita que esta seja utilizada para fabricação ou aplicação em diversos produtos (SHI et al., 2014).

Em bactérias, a produção de celulose foi relacionada à comunicação célula a célula via sinalização de *quorum-sensing* que controla a formação do biofilme. Essa produção ocorre principalmente na interface líquido/ar, geralmente em pH entre 4 e 7 e temperatura entre 25 e 30 ºC. Estima-se que uma única célula de *K. xylinus* possa polimerizar até 200 000 moléculas de glicose em cadeias de glucana por segundo (SALGADO et al., 2019; VALERA et al., 2015). Para o microrganismo que a produz, a película de celulose funciona não somente como um dispositivo de flutuação que mantém as bactérias na superfície rica em oxigênio, mas também é uma proteção das células contra a desidratação, luz solar e radiação ultravioleta, fornecendo os nutrientes necessários através de difusão (AYDIN; AKSOY, 2014; FORESTI; VÁZQUEZ; BOURY, 2017). A formação do polissacarídeo foi relacionada também com a resistência das bactérias a condições estressantes, como elevadas concentrações de ácido acético e etanol. Tem sido demonstrado que o biofilme

produzido por linhagens de BAA serve como uma barreira, impedindo a difusão do ácido acético para a membrana citoplasmática dos microrganismos (AYDIN; AKSOY, 2014; KANCHANARACH et al., 2010; VALERA et al., 2015).

Assim, como outros metabólitos de origem microbiana, a produtividade da biocelulose é influenciada pela composição do meio de cultura e dependente dos nutrientes fornecidos para o crescimento das bactérias, tais como, as fontes de carbono, nitrogênio, fósforo, enxofre, potássio, magnésio e vitaminas (MOHITE; SALUNKE; PATIL, 2013; LEE et al., 2014). Como fonte de carbono, principal ingrediente usado para promover o crescimento e o metabolismo celular durante a síntese de celulose, são utilizados açúcares como a sacarose, glicose e frutose, poliálcoois como o manitol e glicerol, ou ainda resíduos e derivados de alimentos contendo carboidratos. O extrato de levedura, caseína hidrolisada, sulfato de amônio, peptona, glutamato de sódio e glicina são as fontes de nitrogênio geralmente usadas (YIM; SONG; KIM, 2017). Também tem sido observado que a adição de etanol em baixas concentrações fornece energia para a bactéria e promove o crescimento celular no estágio inicial da fermentação, aumentando o rendimento da produção (LI et al., 2012).

O efeito de várias fontes de carbono e nitrogênio para a produção de celulose por *A. xylinus* foi investigado por Ramana, Tomar e Singh (2000) onde observaram que entre as fontes de carbono investigadas, entre estas o sorbitol, glicose, galactose, lactose, sacarose, ácido acético, manitol, maltose e amido; a sacarose, a glicose e o manitol foram consideradas as fontes mais adequadas para obtenção de bom rendimento. Entre as fontes de nitrogênio avaliadas, como a caseína hidrolisada, sulfato de amônio, glicina, farelo de soja, peptona e glutamato de sódio, foi encontrado que a peptona, o sulfato de amônio e a caseína hidrolisada foram as fontes mais adequadas para a síntese de celulose.

Entre os principais meios de cultura relatados para a síntese de biocelulose destacam-se os meios HS (HESTRIN; SCHRAMM, 1954), Yamanaka (1989) e Zhou (2007). O meio HS contém uma pequena quantidade de fonte de carbono, uma fonte de nitrogênio enriquecida (extrato de levedura e peptona) e uma pequena quantidade de ácido cítrico, enquanto que o meio de Yamanaka tem uma grande quantidade de fonte de carbono e é enriquecido com minerais. O meio de Zhou é preparado usando uma combinação dos meios HS e Yamanaka. Além desses, destacam-se também o meio sintético de Mohite, Kamalja e Patil (2012) contendo

NO_3^- como fonte de nitrogênio e o meio sintético de Matsuoka et al. (1996) enriquecido com minerais (potássio, magnésio, ferro, cálcio, molibdênio, zinco e cobre) e vitaminas (inositol, ácido nicotínico, piridoxina, tiamina, ácido pantotênico, riboflavina, ácido p-aminobenzoico, ácido fólico e biotina).

Os efeitos de diferentes macronutrientes como fontes de carbono, nitrogênio, sódio, magnésio, cálcio e etanol sobre a produção de celulose bacteriana com uso de ferramentas estatísticas de otimização foram investigados por diversos autores (JAGANNATH et al., 2008; ZENG; SMALL; WAN, 2011; MOHITE; KAMALJA; PATIL, 2012; BAGEWADI et al., 2020; SANTOSO et al., 2020) sendo alcançados resultados satisfatórios de produção. Nesse contexto, há evidências de que os micronutrientes essenciais como minerais, vitaminas e aminoácidos podem também impactar a produção deste biopolímero pelas espécies produtoras. No entanto, poucos relatos são encontrados abordando este assunto, principalmente com aplicação de ferramentas estatísticas de análise de efeitos e otimização da resposta desejada.

Em seu estudo sobre os efeitos na produção de biocelulose ocasionados pela adição individual de micronutrientes, Ishikawa et al. (1995) verificaram que o ácido nicotínico, ácido p-aminobenzoico, biotina e piridoxina foram as vitaminas mais estimulantes para a produção por *K. sucrofermentans* BPR2001 um meio sintético contendo frutose como fonte de carbono. Son et al. (2003) relataram que a adição individual de ferro, boro e niacina aumentou o nível de produção por uma *Acetobacter* sp. V6 em um meio sintético contendo glicose como fonte de carbono. A investigação das necessidades nutricionais de culturas de *A. melanogenum*, *A. oxydans* e *A. rancens* mostrou que essas espécies necessitaram principalmente de ácido pantotênico, ácido p-aminobenzóico e ácido nicotínico em meio sintético contendo glicose como fonte de carbono (FODA; VAUGHN, 1953). Em outro estudo, Keshk (2014) verificou que a presença de vitamina C no meio HS aumentou o rendimento de BC por todas as linhagens de *Gluconacetobacter* spp. testadas.

A respeito dos efeitos na produção de biocelulose ocasionada pela suplementação do meio de cultura com diferentes aminoácidos adicionados individualmente, Heo e Son (2002) observaram que a omissão de arginina ou treonina afetou a produção causando uma leve diminuição. Da mesma forma, Son et al. (2003) verificaram que a adição de tirosina, valina, metionina, isoleucina ou glicina reduziu o nível de biocelulose por *Acetobacter* sp. V6. No entanto, a adição de outros aminoácidos aumentou ligeiramente a produção. Em seu estudo,

Matsuoka et al. (1996) verificaram que a metionina estimulou a taxa de crescimento durante o estágio inicial do período de cultivo e aumentou a produção de celulose por *A. sucrofermentans*. O estudo sugere que metionina seria um aminoácido essencial para a obtenção de altos rendimento de biocelulose e estimulante do crescimento celular para esta cepa.

Além do estudo nutricional dos microrganismos, ressalta-se também a importância de investigar novas formas de cultivo que podem impactar no rendimento do produto de interesse. Ressalta-se ainda a necessidade de avaliar as características e propriedades dos materiais obtidos nas condições estudadas considerando-se que estes fatores podem influenciar nas aplicações específicas das membranas de celulose bacteriana desenvolvidas nos estudos.

4.1.5. Referências

ADEBAYO-OYETORO, A. O.; ADENUBI, E.; OGUNDIPE, O. O.; BANKOLE, B. O.; ADEYEYE, S. A. O. Production and quality evaluation of vinegar from mango. **Cogent Food & Agriculture,** v. 3, n. 1, p. 1278193, 2017. https://doi.org/10.1080/23311932.2016.1278193

AHMED, J.; GULTEKINOGLU, M.; EDIRISINGHE, M.. Bacterial cellulose micro-nano fibres for wound healing applications. **Biotechnology Advances,** v. 41, p. 107549, jul./ago. 2020. https://doi.org/10.1016/j.biotechadv.2020.107549

AJE, O. F.; ADIE, A. J. Response surface methodology and Taguchi method based applications–A Review. **Global Journal of Engineering and Technology Advances,** v. 5, n. 1, p. 053-059, 2020. https://doi.org/10.30574/gjeta.2020.5.1.0062

AKRAM, M.; ASIF, H. M.; UZAIR, M.; AKHTAR, N.; MADNI, A.; SHAH, S. A.; HASAN, Z.; ULLAH, A. Amino acids: A review article. **Journal of Medicinal Plants Research**, v. 5, n. 17, p. 3997-4000, set. 2011. https://doi.org/10.5897/JMPR.9000061

ANDRIANI, D.; APRIYANA, A. Y.; KARINA, M. The optimization of bacterial cellulose production and its applications: a review. **Cellulose,** v. 27, p. 6747-6766, ago. 2020. https://doi.org/10.1007/s10570-020-03273-9

ANDRÉS-BARRAO, C.; BENAGLI, C.; CHAPPUIS, M.; PÉREZ, R. O.; TONOLLA, M.; BARJA, F. Rapid identification of acetic acid bacteria using MALDI-TOF mass

spectrometry fingerprinting. **Systematic and Applied Microbiology,** v. 36, n. 2, p. 75-81, mar. 2013. https://doi.org/10.1016/j.syapm.2012.09.002

ANDRÉS-BARRAO, C.; SAAD, M. M.; FERRETE, E. C., BRAVO, D.; CHAPPUIS, M.-L.; PÉREZ, R. O.; JUNIER, P.; PERRET, X.; BARJA, F. Metaproteomics and ultrastructure characterization of *Komagataeibacter* spp. involved in high-acid spirit vinegar production. **Food Microbiology,** v. 55, p. 112-122, maio 2016. https://doi.org/10.1016/j.fm.2015.10.012

ASAI, T. **Acetic acid bacteria classification and biochemical activities**. Tokyo: University of Tokyo, 1968. 343 p.

AUGIMERI, R. V.; STRAP, J. L. The phytohormone ethylene enhances cellulose production, regulates CRP/FNRKx transcription and causes differential gene expression within the bacterial cellulose synthesis operon of *Komagataeibacter* (*Gluconacetobacter*) *xylinus* ATCC 53582. **Frontiers in Microbiology,** v. 6, p. 1459, dez. 2015. https://doi.org/10.3389/fmicb.2015.01459

AWUCHI, C. G.; IGWE, V. S.; AMAGWULA, I. O.; ECHETA, C. K. Health benefits of micronutrients (vitamins and minerals) and their Associated Deficiency Diseases: a systematic review. **International Journal of Food Sciences,** v. 3, n. 1, p. 1-32, 2020. https://www.iprjb.org/journals/index.php/IJF/article/view/1024

AYDIN, Y. A.; AKSOY, N. D. Isolation and characterization of an efficient bacterial cellulose producer strain in agitated culture: *Gluconacetobacter hansenii* P2A. **Applied Microbiology and Biotechnology,** v. 98, n. 3, p. 1065-1075, fev. 2014. https://doi.org/10.1007/s00253-013-5296-9

AZAD, Sabina. Amino Acids: Its types and uses. **International Journal of Clinical and Diagnostic Pathology**, v. 1, n. 1, p. 10-13, 2018. https://www.patholjournal.com/articles/4/1-1-3-181.pdf

AZEREDO, H.; BARUD, H.; FARINAS, C. S.; VASCONCELLOS, V. M.; CLARO, A. M. Bacterial cellulose as a raw material for food and food packaging applications. **Frontiers in Sustainable Food Systems,** v. 3, p. 7, fev. 2019. https://doi.org/10.3389/fsufs.2019.00007

BADSHAH, M.; ULLAH, H.; KHAN, A. R.; KHAN, S.; PARK, J. K.; KHAN, T. Surface modification and evaluation of bacterial cellulose for drug delivery. **International Journal of Biological Macromolecules,** v. 113, p. 526-533, jul. 2018. https://doi.org/10.1016/j.ijbiomac.2018.02.135

BAGEWADI, Z. K.; BHAVIKATTI, J. S.; MUDDAPUR, U. M.; YARAGUPPI, D. A.; MULLA, S. I. Statistical optimization and characterization of bacterial cellulose produced by isolated thermophilic *Bacillus licheniformis* strain ZBT2. **Carbohydrate Research,** v. 491, p. 107979, maio 2020. https://doi.org/10.1016/j.carres.2020.107979

BANY SAYD, A. A. K. Amino Acids changes during citrus storage. **Food Science and Quality Management,** v. 24, p. 61-69, 2014. https://core.ac.uk/download/pdf/234683765.pdf

BAYIR, E.; BILGI, E.; HAMES, E. E.; SENDEMIR, A. Production of hydroxyapatite–bacterial cellulose composite scaffolds with enhanced pore diameters for bone tissue engineering applications. **Cellulose,** v. 26, n. 18, p. 9803-9817, dez. 2019. https://doi.org/10.1007/s10570-019-02763-9

BEHERA, S. K.; MEENA, H.; CHAKRABORTY, S.; MEIKAP, B. C. Application of response surface methodology (RSM) for optimization of leaching parameters for ash reduction from low-grade coal. **International Journal of Mining Science and Technology,** v. 28, n. 4, p. 621-629, jul. 2018. https://doi.org/10.1016/j.ijmst.2018.04.014

BERINGHS, A. O.; DALMINA, M.; CRECZYNSKI-PASA, T. B.; SONAGLIO, D. Response Surface Methodology IV-Optimal design applied to the performance improvement of an RP-HPLC-UV method for the quantification of phenolic acids in Cecropia glaziovii products. **Revista Brasileira de Farmacognosia,** v. 25, n. 5, p. 513-521, set./out. 2015. https://doi.org/10.1016/j.bjp.2015.05.007

BILGI, E.; BAYIR, E.; SENDEMIR-URKMEZ, A.; HAMES, E. E. Optimization of bacterial cellulose production by *Gluconacetobacter xylinus* using carob and haricot bean. **International Journal of Biological Macromolecules,** v. 90, p. 2-10, set. 2016. https://doi.org/10.1016/j.ijbiomac.2016.02.052

BLANCO PARTE, F. G.; SANTOSO, S. P.; CHOU, C. C.; VERMA, V.; WANG, H. T.; ISMADJI, S.; CHENG, K. C. Current progress on the production, modification, and applications of bacterial cellulose. **Critical Reviews in Biotechnology,** v. 40, n. 3, p. 397-414, 2020. https://doi.org/10.1080/07388551.2020.1713721

BONNET, M.; LAGIER, J. C.; RAOULT, D.; KHELAIFIA, S. Bacterial culture through selective and non-selective conditions: the evolution of culture media in clinical microbiology. **New Microbes And New Infections,** v. 34, p. 100622, mar. 2020. https://doi.org/10.1016/j.nmni.2019.100622

BOUDRAHEM, F.; ZIANI, S.; AISSANI-BENISSAD, F. Application of response surface methodology based on central composite design for optimization of yellow bezacryl sorption on shoe soles waste. **Environmental Progress & Sustainable Energy,** v. 38, n. s1, p. S412-S421, mar. 2019. https://doi.org/10.1002/ep.13073

CACICEDO, M. L.; CASTRO, M. C.; SERVETAS, I.; BOSNEA, L.; BOURA, K.; TSAFRAKIDOU, P.; DIMA, A.; TERPOU, A.; KOUTINAS, A.; CASTRO, G. R. Progress in bacterial cellulose matrices for biotechnological applications. **Bioresource Technology,** v. 213, p. 172-180, ago. 2016. https://doi.org/10.1016/j.biortech.2016.02.071

CACICEDO, M. L.; ISLAN, G. A.; DRACHEMBERG, M. F.; ALVAREZ, V. A.; BARTEL, L. C.; BOLZÁN, A. D.; CASTRO, G. R. Hybrid bacterial cellulose–pectin films for delivery of bioactive molecules. **New Journal of Chemistry,** v. 42, n. 9, p. 7457-7467, 2018. https://doi.org/10.1039/C7NJ03973E

CAMPANO, C.; MERAYO, N.; NEGRO, C.; BLANCO, A. In situ production of bacterial cellulose to economically improve recycled paper properties. **International Journal of Biological Macromolecules,** v. 118, p. 1532-1541, out. 2018. https://doi.org/10.1016/j.ijbiomac.2018.06.201

CAMPANO, C.; BALEA, A.; BLANCO, A.; NEGRO, C. Enhancement of the fermentation process and properties of bacterial cellulose: A review. **Cellulose,** v. 23, n. 1, p. 57-91, fev. 2016. https://doi.org/10.1007/s10570-015-0802-0

CAMPBELL, Iain. Macronutrients, minerals, vitamins and energy. **Anaesthesia & Intensive Care Medicine,** v. 18, n. 3, p. 141-146, mar. 2017. https://doi.org/10.1016/j.mpaic.2016.11.014

CAZÓN, P.; VÁZQUEZ, M. Improving bacterial cellulose films by ex-situ and in-situ modifications: a review. **Food Hydrocolloids,** v.113, p. 106514, abr. 2021. https://doi.org/10.1016/j.foodhyd.2020.106514

CHANDRASEKARAN, P. T.; BARI, N. K.; SINHA, S. Enhanced bacterial cellulose production from *Gluconobacter xylinus* using super optimal broth. **Cellulose,** v. 24, n. 10, p. 4367-4381, out. 2017. https://doi.org/10.1007/s10570-017-1419-2

CHEN, S.-Q.; MIKKELSEN, D.; LOPEZ-SANCHEZ, P.; WANG, D.; MARTINEZ-SANZ, M.; GILBERT, E. P.; FLANAGAN, B. M.; GIDLEY, M. J. Characterisation of bacterial cellulose from diverse *Komagataeibacter* strains and their application to construct plant cell wall analogues. **Cellulose,** v. 24, n. 3, p. 1211-1226, mar. 2017. https://doi.org/10.1007/s10570-017-1203-3

CHINA, S. L.; ZANICHELLI, G.; DE VERO, L.; GULLO, M. Oxidative fermentations and exopolysaccharides production by acetic acid bacteria: a mini review. **Biotechnology Letters,** v. 40, n. 9, p. 1289-1302, out. 2018. https://doi.org/10.1007/s10529-018-2591-7

CHUAH, C.; WANG, J.; TAVAKOLI, J.; TANG, Y. Novel bacterial cellulose-poly (acrylic acid) hybrid hydrogels with controllable antimicrobial ability as dressings for chronic wounds. **Polymers,** v. 10, n. 12, p. 1323, 2018. https://doi.org/10.3390/polym10121323

CHUNSHOM, N.; CHUYSINUAN, P.; TECHASAKUL, S.; UMMARTYOTIN, S. Dried-state bacterial cellulose (*Acetobacter xylinum*) and polyvinyl-alcohol-based hydrogel: An approach to a personal care material. **Journal of Science: Advanced Materials and Devices,** v. 3, n. 3, p. 296-302, set. 2018. https://doi.org/10.1016/j.jsamd.2018.06.004

COMBS JR, G. F.; MCCLUNG, J. P. **The vitamins: fundamental aspects in nutrition and health**. Academic press, 2016.

COSTA, A. F. S.; ALMEIDA, F. C. G.; VINHAS, G. M.; SARUBBO, L. A. Production of bacterial cellulose by *Gluconacetobacter hansenii* using corn steep liquor as nutrient sources. **Frontiers in Microbiology,** v. 8, p. 1-12, out. 2017. https://doi.org/10.3389/fmicb.2017.02027

COSTA-PINTO, R.; GANTNER, D. Macronutrients, minerals, vitamins and energy. **Anaesthesia & Intensive Care Medicine**, v. 21, n. 3, p. 157-161, mar. 2020. https://doi.org/10.1016/j.mpaic.2019.12.006

DAĞBAĞLI, S.; GÖKSUNGUR, Y. Exopolysaccharide production of acetic acid bacteria. In: SENGUN, I. Y. **Acetic acid bacteria:** Fundamentals and food applications. Boca Raton: CRC Press, 2017. p. 120-141.

DE ROOS, J.; DE VUYST, L. Acetic acid bacteria in fermented foods and beverages. **Current Opinion in Biotechnology,** v. 49, p. 115-119, fev. 2018. https://doi.org/10.1016/j.copbio.2017.08.007

DINARVAND, M.; REZAEE, M.; FOROUGHI, M. Optimizing culture conditions for production of intra and extracellular inulinase and invertase from *Aspergillus niger* ATCC 20611 by response surface methodology (RSM). **Brazilian Journal of Microbiology,** v. 48, n. 3, p. 427-441, jul./set. 2017. http://doi.org/10.1016/j.bjm.2016.10.026

DING, R.; HU, S.; XU, M.; HU, Q.; JIANG, S.; XU, K.; TREMBLAY, P.-L.; ZHANG, T. The facile and controllable synthesis of a bacterial cellulose/polyhydroxybutyrate

composite by co-culturing *Gluconacetobacter xylinus* and *Ralstonia eutropha*. **Carbohydrate Polymers,** v. 252, p. 117137, jan. 2021. https://doi.org/10.1016/j.carbpol.2020.117137

EJAZ, U.; AHMED, A.; SOHAIL, M. Statistical optimization of immobilization of yeast cells on corncob for pectinase production. **Biocatalysis and Agricultural Biotechnology,** v. 14, p. 450-456, abr. 2018. https://doi.org/10.1016/j.bcab.2018.04.011

ESKANDARI, T.; NIAZI, A.; FATEMI, M. H.; CHAICHI, M. J. Determination of azinphos-methyl in environmental samples using alkaline hydrolysis combined with spectroflourimetry and response surface modelling. **International Journal of Environmental Analytical Chemistry,** p. 1-12, 2020. https://doi.org/10.1080/03067319.2020.1714040

ESLAHI, N.; MAHMOODI, A.; MAHMOUDI, N.; ZANDI, N.; SIMCHI, A. Processing and properties of nanofibrous bacterial cellulose-containing polymer composites: A review of recent advances for biomedical applications. **Polymer Reviews,** v. 60, n. 1, p. 144-170, 2020. https://doi.org/10.1080/15583724.2019.1663210

FENNEMA, O. R.; DAMODARAN, S.; PARKIN, K. L. **Fennema's Food Chemistry.** CRC press, 2017.

FERNANDES, I. D. A. A.; PEDRO, A. C.; RIBEIRO, V. R.; BORTOLINI, D. G.; OZAKI, M. S. C.; MACIEL, G. M.; HAMINIUK, C. W. I. Bacterial cellulose: From production optimization to new applications. **International Journal of Biological Macromolecules,** v. 164, p. 2598-2611, dez. 2020. https://doi.org/10.1016/j.ijbiomac.2020.07.255

FERREIRA, S. L.; CAIRES, A. O.; BORGES, T. D. S.; LIMA, A. M.; SILVA, L. O.; SANTOS, W. N. Robustness evaluation in analytical methods optimized using experimental designs. **Microchemical Journal,** v. 131, p. 163-169, mar. 2017. https://doi.org/10.1016/j.microc.2016.12.004

FODA, I. O.; VAUGHN, R. H. The nutritional requirements of *Acetobacter melanogenum* and related species. **Journal of Bacteriology,** v. 65, n. 1, p. 79-82, jan. 1953.

FONTES, M. L.; MENEGUIN, A. B.; TERCJAK, A.; GUTIERREZ, J.; CURY, B. S. F.; SANTOS, A. M.; RIBEIRO, S. J. L.; BARUD, H. S. Effect of in situ modification of bacterial cellulose with carboxymethylcellulose on its nano/microstructure and methotrexate release properties. **Carbohydrate Polymers,** v. 179, p. 126-134, jan. 2018. https://doi.org/10.1016/j.carbpol.2017.09.061

FORESTI, M. L.; VÁZQUEZ, A.; BOURY, B. Applications of bacterial cellulose as precursor of carbon and composites with metal oxide, metal sulfide and metal nanoparticles: A review of recent advances. **Carbohydrate Polymers,** v. 157, p. 447-467, fev. 2017. https://doi.org/10.1016/j.carbpol.2016.09.008

FUKUDA, I. M.; PINTO, C. F. F.; MOREIRA, C. D. S.; SAVIANO, A. M.; LOURENÇO, F. R. Design of experiments (DoE) applied to pharmaceutical and analytical quality by design (QbD). **Brazilian Journal of Pharmaceutical Sciences,** v. 54, n. SPE, nov. 2018. http://doi.org/10.1590/s2175-97902018000001006

GAO, J. X.; LI, P.; DU, X. J.; HAN, Z. H.; XUE, R.; LIANG, B., WANG, S. A negative regulator of Cellulose biosynthesis, bcsR, affects biofilm formation, and adhesion/invasion ability of Cronobacter sakazakii. **Frontiers in Microbiology,** v. 8, p. 1839, set. 2017. https://doi.org/10.3389/fmicb.2017.01839

GAO, G.; CAO, Y.; ZHANG, Y.; WU, M.; MA, T.; LI, G. In situ production of bacterial cellulose/xanthan gum nanocomposites with enhanced productivity and properties using Enterobacter sp. FY-07. **Carbohydrate Polymers,** v. 248, p. 116788, nov. 2020. https://doi.org/10.1016/j.carbpol.2020.116788

GORGIEVA, S; TRČEK, J. Bacterial cellulose: Production, modification and perspectives in biomedical applications. **Nanomaterials,** v. 9, n. 10, p. 1352, 2019. https://doi.org/10.3390/nano9101352

GIBSON, L. J. The hierarchical structure and mechanics of plant materials. **Journal of the Royal Society Interface,** v. 12, n. 9, p. 2749-2766, set. 2012. https://doi.org/10.1098/rsif.2012.0341

GOMES, R. J.; BORGES, M. F.; ROSA, M. F.; CASTRO-GÓMEZ, R. J. H.; SPINOSA, W. A. Acetic acid bacteria in the food industry: Systematics, characteristics and applications. **Food Technology and Biotechnology,** v. 56, n. 2, p. 139-151, abr./jun. 2018. https://doi.org/10.17113/ftb.56.02.18.5593

GOMES, R. J.; FARIA-TISCHER, P. C. S.; TISCHER, C. A., CONSTANTINO, L. V.; ROSA, M. F.; CHIDEROLI, R. T.; PEREIRA, U. P.; SPINOSA, W. A. *Komagataeibacter intermedius* V-05: an acetic acid bacterium isolated from vinegar industry, with high capacity for bacterial cellulose production in soybean molasses medium. **Food Technology and Biotechnology,** v. 59, n. 4, out./dez. 2021. https://doi.org/10.17113/ftb.59.04.21.7148

GRAHOVAC, J.; GRAHOVAC, M.; DODIĆ, J.; BAJIĆ, B.; BALAŽ, J. Optimization of cultivation medium for enhanced production of antifungal metabolites by *Streptomyces hygroscopicus*. **Crop Protection,** v. 65, p. 143-152, nov. 2014. https://doi.org/10.1016/j.cropro.2014.07.020

GÜNDOĞDU, T. K.; DENIZ, I.; ÇALIŞKAN, G.; ŞAHIN, E. S.; AZBAR, N. Experimental design methods for bioengineering applications. **Critical Reviews in Biotechnology,** v. 36, n. 2, p. 368-388, 2016. https://doi.org/10.3109/07388551.2014.973014

GÜZEL, M.; AKPINAR, Ö. Production and characterization of bacterial cellulose from citrus peels. **Waste and Biomass Valorization,** v. 10, n. 8, p. 2165-2175, ago. 2019. https://doi.org/10.1007/s12649-018-0241-x

HA, J. H.; SHAH, N.; UL-ISLAM, M.; KHAN, T.; PARK, J. K. Bacterial cellulose production from a single sugar α-linked glucuronic acid-based oligosaccharide. **Process Biochemistry,** v. 46, n. 9, p. 1717-1723, set. 2011. https://doi.org/10.1016/j.procbio.2011.05.024

HAMMAMI, A.; BAYOUDH, A.; HADRICH, B.; ABDELHEDI, O.; JRIDI, M.; NASRI, M. Response-surface methodology for the production and the purification of a new H2O2-tolerant alkaline protease from *Bacillus invictae* AH1 strain. **Biotechnology Progress,** v. 36, n. 3, p. e2965, mai./jun. 2020. https://doi.org/10.1002/btpr.2965

HE, W.; HUANG, X.; ZHENG, Y.; SUN, Y.; XIE, Y.; WANG, Y.; YUE, L. In situ synthesis of bacterial cellulose/copper nanoparticles composite membranes with long-term antibacterial property. **Journal of Biomaterials Science, Polymer Edition,** v. 29, n. 17, p. 2137-2153, 2018. https://doi.org/10.1080/09205063.2018.1528518

HEO, M.-S.; SON, H.-J. Development of an optimized, simple chemically defined medium for bacterial cellulose production by *Acetobacter* sp. A9 in shaking cultures. **Biotechnology and applied biochemistry,** v. 36, n. 1, p. 41-45, ago. 2002. https://doi.org/10.1042/BA20020018

HESTRIN, S.; SCHRAMM, M. Synthesis of cellulose by *Acetobacter xylinum*. 2. Preparation of freeze-dried cells capable of polymerizing glucose to cellulose. **Biochemistry Journal,** v. 58, n. 2, p. 345-352, out. 1954.

HO, C. W.; LAZIM, A. M.; FAZRY, S.; ZAKI, U. K. H. H.; LIM, S. J. Varieties, production, composition and health benefits of vinegars: A review. **Food Chemistry,** v. 221, p. 1621-1630, abr. 2017. https://doi.org/10.1016/j.foodchem.2016.10.128

HOSNY, K. M.; AHMED, O. A.; FAHMY, U. A.; ALKHALIDI, H. M. Nanovesicular systems loaded with a recently approved second generation type-5 phospodiesterase inhibitor (avanafil): I. Plackett-Burman screening and characterization. **Journal of Drug Delivery Science and Technology,** v. 43, p. 154-159, fev. 2018. https://doi.org/10.1016/j.jddst.2017.10.009

HUANG, H.-C.; CHEN, L.-C.; LIN, S.-B.; HSU, C.-P.; CHEN, H.-H. *In situ* modification of bacterial cellulose network structure by adding interfering substances during fermentation. **Bioresource Technology,** v. 101, n. 15, p. 6084-6091, ago. 2010. https://doi.org/10.1016/j.biortech.2010.03.031

HUANG, Y.; ZHU, C.; YANG, J.; NIE, Y.; CHEN, C.; SUN, D. Recent advances in bacterial cellulose. **Cellulose,** v. 21, n. 1, p. 1-30, fev. 2014. https://doi.org/10.1007/s10570-013-0088-z

IGUCHI, M.; YAMANAKA, S.; BUDHIONO, A. Bacterial cellulose—a masterpiece of nature's arts. **Journal of Materials Science,** v. 35, n. 2, p. 261-270, jan. 2000. https://doi.org/10.1023/A:1004775229149

ISHIKAWA, A.; MATSUOKA, M.; TSUCHIDA, T.; YOSHINAGA, F. Increase in cellulose production by sulfaguanidine-resistant mutants derived from *Acetobacter xylinum* subsp. *sucrofermentans*. **Journal Bioscience, Biotechnology, and Biochemistry,** v. 59, n. 12, p. 2259-2262, 1995. https://doi.org/10.1271/bbb.59.2259

ISLAM, M. U.; ULLAH, M. W.; KHAN, S.; SHAH, N.; PARK, J. K. Strategies for cost-effective and enhanced production of bacterial cellulose. **International Journal of Biological Macromolecules,** v. 102, p. 1166-1173, set. 2017. https://doi.org/10.1016/j.ijbiomac.2017.04.110

JACYNA, J.; KORDALEWSKA, M.; MARKUSZEWSKI, M. J. Design of Experiments in metabolomics-related studies: An overview. **Journal of Pharmaceutical and Biomedical Analysis,** v. 164, p. 598-606, fev. 2019. https://doi.org/10.1016/j.jpba.2018.11.027

JAGANNATH, A.; KALAISELVAN, A.; MANJUNATHA, S. S.; RAJU, P. S.; BAWA, A. S. The effect of pH, sucrose and ammonium sulphate concentrations on the production of bacterial cellulose (Nata-de-coco) by Acetobacter xylinum. **World Journal of Microbiology and Biotechnology,** v. 24, n. 11, p. 2593-2599, 2008. https://doi.org/10.1007/s11274-008-9781-8

JAWAN, R.; ABBASILIASI, S.; TAN, J. S.; MUSTAFA, S.; HALIM, M.; ARIFF, A. B. Influence of culture conditions and medium compositions on the production of bacteriocin-like inhibitory substances by *Lactococcus lactis* Gh1. **Microorganisms,** v. 8, n. 10, p. 1454, 2020. https://doi.org/10.3390/microorganisms8101454

JIA, Y.; WANG, X.; HUO, M.; ZHAI, X.; LI, F.; ZHONG, C. Preparation and characterization of a novel bacterial cellulose/chitosan bio-hydrogel. **Nanomaterials and Nanotechnology,** v. 7, p. 1-8, 2017. https://doi.org/10.1177/1847980417707172

JO, J. H.; LEE, D. S.; PARK, D.; CHOE, W.-S.; PARK, J. M. Optimization of key process variables for enhanced hydrogen production by *Enterobacter aerogenes* using statistical methods. **Bioresource Technology,** v. 99, n. 6, p. 2061-2066, abr. 2008. https://doi.org/10.1016/j.biortech.2007.04.027

JOZALA, A. F.; PÉRTILE, R. A. N.; SANTOS, C. A.; SANTOS-EBINUMA, V. C.; SECKLER, M. M.; GAMA, F. M.; PESSOA, A. Bacterial cellulose production by Gluconacetobacter xylinus by employing alternative culture media. **Applied Microbiology and Biotechnology,** v. 99, n. 3, p. 1181-1190, fev. 2015. https://doi.org/10.1007/s00253-014-6232-3

JU, Y.; SON, K. H.; JIN, C.; HWANG, B. S.; PARK, D. J.; KIM, C. J. Statistical optimization of culture medium for improved production of antimicrobial compound by *Streptomyces rimosus* AG-P1441. **Food Science and Biotechnology,** v. 27, n. 2, p. 581-590, abr. 2018. https://doi.org/10.1007/s10068-017-0257-1

KANCHANARACH, W.; THEERAGOOL, G.; INOUE, T.; YAKUSHI, T.; ADACHI, O.; MATSUSHITA, K. Acetic acid fermentation of *Acetobacter pasteurianus*: Relationship between acetic acid resistance and pellicle polysaccharide formation. **Bioscience, Biotechnology, and Biochemistry,** v. 74, n. 8, p. 1591-1597, 2010. https://doi.org/10.1271/bbb.100183

KENNEDY, M.; KROUSE, D. Strategies for improving fermentation medium performance: A review. **Journal of Industrial Microbiology and Biotechnology,** v. 23, n. 6, p. 456-475, dez. 1999. https://doi.org/10.1038/sj.jim.2900755

KESHK, S. M. A. S. Vitamin C enhances bacterial cellulose production in *Gluconacetobacter xylinus*. **Carbohydrate polymers,** v. 99, p. 98-100, jan. 2014. https://doi.org/10.1016/j.carbpol.2013.08.060

KESKIN, Z.; URKMEZ, A. S.; HAMES, E. E. Novel keratin modified bacterial cellulose nanocomposite production and characterization for skin tissue engineering. **Materials Science and Engineering: C,** v. 75, p. 1144-1153, jun. 2017. https://doi.org/10.1016/j.msec.2017.03.035

KHALID, A.; KHAN, R.; UL-ISLAM, M.; KHAN, T.; WAHID, F. Bacterial cellulose-zinc oxide nanocomposites as a novel dressing system for burn wounds. **Carbohydrate Polymers,** v. 164, p. 214-221, maio 2017. https://doi.org/10.1016/j.carbpol.2017.01.061

KIM, J. H.; PARK, S.; KIM, H.; KIM, H. J.; YANG, Y.-H.; KIM, Y. H.; JUNG, S.-K.; KAN, E.; LEE, S. H. Alginate/bacterial cellulose nanocomposite beads prepared using *Gluconacetobacter xylinus* and their application in lipase immobilization.

Carbohydrate Polymers, v. 157, p. 137-145, fev. 2017. https://doi.org/10.1016/j.carbpol.2016.09.074

KOECH, J. K.; MUTISO, M. J.; KOSKEI, J. K. Response surface methodology approach to the optimization of potato (*Solanum tuberosum*) tuber yield using second-order rotatable design. **Journal of Biometrics & Biostatistics,** v. 8, n. 3, p. 341, 2017. https://doi.org/10.4172/2155-6180.1000351

KOUR, R.; JAIN, D.; BHOJIYA, A. A.; SUKHWAL, A.; SANADHYA, S.; SAHEEWALA, H.; JAT, G.; SINGH, A.; MOHANTY, S. R. Zinc biosorption, biochemical and molecular characterization of plant growth-promoting zinc-tolerant bacteria. **3 Biotech,** v. 9, n. 11, p. 1-17, out. 2019. https://doi.org/10.1007/s13205-019-1959-2

KUMBHAR, J. V.; RAJWADE, J. M.; PAKNIKAR, K. M. Fruit peels support higher yield and superior quality bacterial cellulose production. **Applied Microbiology and Biotechnology**, v. 99, n. 16, p. 6677-6691, ago. 2015. https://doi.org/10.1007/s00253-015-6644-8

KUROSUMI, A.; SASAKI, C.; YAMASHITA, Y.; NAKAMURA, Y. Utilization of various fruit juices as carbon source for production of bacterial cellulose by Acetobacter xylinum NBRC 13693. **Carbohydrate Polymers,** v. 76, n. 2, p. 333-335, mar. 2009. https://doi.org/10.1016/j.carbpol.2008.11.009

LEE, K.-Y.; BULDUM, G.; MANTALARIS, A.; BISMARCK, A. More than meets the eye in bacterial cellulose: Biosynthesis, bioprocessing, and applications in advanced fiber composites. **Macromolecular Bioscience,** v. 14, n. 1, p. 10-32, jan. 2014. https://doi.org/10.1002/mabi.201300298

LEE, S. E.; PARK, Y. S. The role of bacterial cellulose in artificial blood vessels. **Molecular & Cellular Toxicology,** v. 13, n. 3, p. 257-261, set. 2017. https://doi.org/10.1007/s13273-017-0028-3

LI, Y.; TIAN, C.; TIAN, H.; ZHANG, J.; HE, X.; PING, W.; LEI, H. Improvement of bacterial cellulose production by manipulating the metabolic pathways in which ethanol and sodium citrate involved. **Applied Microbiology and Biotechnology,** v. 96, n. 6, p. 1479-1487, dez. 2012. https://doi.org/10.1007/s00253-012-4242-6

LIN, S.-P.; CALVAR, I. L.; CATCHMARK, J. M.; LIU, J.-R.; DEMIRCI, A.; CHENG, K.-C. Biosynthesis, production and applications of bacterial cellulose. **Cellulose,** v. 20, n. 5, p. 2191-2219, out. 2013. https://doi.org/10.1007/s10570-013-9994-3

LIN, S.-P.; LIU, C.-T.; HSU, K.-D.; HUNG, Y.-T.; SHIH, T.-Y.; CHENG, K.-C. Production of bacterial cellulose with various additives in a PCS rotating disk bioreactor and its material property analysis**. Cellulose,** v. 23, n. 1, p. 367-377, fev. 2016. https://doi.org/10.1007/s10570-015-0855-0

LIN, D.; LIU, Z.; SHEN, R.; CHEN, S.; YANG, X. Bacterial cellulose in food industry: Current research and future prospects. **International Journal of Biological Macromolecules,** v. 158, p. 1007-1019, set. 2020. https://doi.org/10.1016/j.ijbiomac.2020.04.230

LIU, W.; DU, H.; ZHANG, M.; LIU, K.; LIU, H.; XIE, H.; ZHANG X.; SI, C. Bacterial cellulose-based composite scaffolds for biomedical applications: a review. **ACS Sustainable Chemistry & Engineering**, v. 8, n. 20, p. 7536-7562, abr. 2020. https://doi.org/10.1021/acssuschemeng.0c00125

LO, K.-J.; LEE, S.-K.; LIU, C.-T. Development of a low-cost culture medium for the rapid production of plant growth-promoting *Rhodopseudomonas palustris* strain PS3. **Plos One,** v. 15, n. 7, p. e0236739, jul. 2020. https://doi.org/10.1371/journal.pone.0236739

LOUHICHI, G., BOUSSELMI, L., GHRABI, A., & KHOUNI, I. Process optimization via response surface methodology in the physico-chemical treatment of vegetable oil refinery wastewater. **Environmental Science and Pollution Research**, v. 26, n. 19, p. 18993-19011, jul. 2019. https://doi.org/10.1007/s11356-018-2657-z

LOTFIMAN, S.; BIAK, D. R. A.; TI, T. B.; KAMARUDIN, S.; NIKBIN, S. Influence of date syrup as a carbon source on bacterial cellulose production by *Acetobacter xylinum* 0416. **Advances in Polymer Technology,** v. 37, n. 4, p. 1085-1091, jun. 2018. https://doi.org/10.1002/adv.21759

LU, S.; MIKKELSEN, D.; FLANAGAN, B. M.; WILLIAMS, B. A.; GIDLEY, M. J. Interaction of cellulose and xyloglucan influences in vitro fermentation outcomes. **Carbohydrate Polymers,** v. 258, p. 117698, abr. 2021. https://doi.org/10.1016/j.carbpol.2021.117698

LUO, H.; DONG, J.; YAO, F.; YANG, Z.; LI, W.; WANG, J.; XU, X.; HU, J.; WAN, Y. Layer-by-layer assembled bacterial cellulose/graphene oxide hydrogels with extremely enhanced mechanical properties. **Nano-Micro Letters,** v. 10, n. 3, p. 1-10, 2018. https://doi.org/10.1007/s40820-018-0195-3

LUTHRA, U.; SINGH, N.; TRIPATHI, A.; VORA, S.; BHOSLE, V. Media optimization for lovastatin production by statistical approach using *Aspergillus terreus* by submerged fermentation. **Journal of Medical Science And clinical Research,** v. 3, n. 2, p. 4520-4528, fev. 2015.

LYNCH, K. M.; ZANNINI, E.; WILKINSON, S.; DAENEN, L.; ARENDT, E. K. Physiology of acetic acid bacteria and their role in vinegar and fermented beverages. **Comprehensive Reviews in Food Science and Food Safety,** v. 18, n. 3, p. 587-625, maio 2019. https://doi.org/10.1111/1541-4337.12440

MACHADO, R. T.; MENEGUIN, A. B.; SÁBIO, R. M.; FRANCO, D. F.; ANTONIO, S. G.; GUTIERREZ, J.; TERCJAK, A.; BERRETTA, A. A.; RIBEIRO, S. J. L.; LAZARINI, S. C.; LUSTRI, W. R.; BARUD, H. S. Komagataeibacter rhaeticus grown in sugarcane molasses-supplemented culture medium as a strategy for enhancing bacterial cellulose production. **Industrial Crops and Products,** v. 122, p. 637-646, out. 2018. https://doi.org/10.1016/j.indcrop.2018.06.048

MADIGAN, M. T.; MARTINKO, J. M.; BENDER, K. S.; BUCKLEY, D. H.; STAHL, D. A. **Microbiologia de Brock.** 14 ed. Porto Alegre: Artmed, 2016.

MANDENIUS, C.-F.; BRUNDIN, A. Bioprocess optimization using design-of-experiments methodology. **Biotechnology Progress,** v. 24, n. 6, p. 1191-1203, nov./dez. 2008. https://doi.org/10.1002/btpr.67

MARESTONI, L. D.; BARUD, H. D. S.; GOMES, R. J.; CATARINO, R. P. F.; HATA, N. N. Y.; RESSUTTE, J. B.; SPINOSA, W. A. Commercial and potential applications of bacterial cellulose in Brazil: ten years review. **Polímeros,** v. 30, n. 4, 2020. http://doi.org/10.1590/0104-1428.09420

MATSUOKA, M.; TSUCHIDA, T.; MATSUSHITA, K.; ADACHI, O.; YOSHINAGA, F. A synthetic medium for bacterial cellulose production by *Acetobacter xylinum* subsp. *Sucrofermentans*. **Bioscience, Biotechnology, and Biochemistry,** v. 60, n. 4, p. 575-579, jan. 1996. https://doi.org/10.1271/bbb.60.575

MEDEIROS, V. D. N.; SILVA, B. I. A.; FERREIRA, R. D. S. B.; OLIVEIRA, S. S. L.; DIAS, R. A.; ARAÚJO, E. M. Optimization of Process Parameters for Obtaining Polyethersulfone/Additives Membranes. **Water,** v. 12, n. 8, p. 2180, 2020. https://doi.org/10.3390/w12082180

MOBEEN, S.; SANKAR, G. Bioprocess development employing design of experiments for antibiotic production from Streptomyces parvulus strain Sankarensis-A10. **Indian Journal of Pharmaceutical Sciences,** v. 80, n. 5, p. 911-920, set./out. 2018. https://doi.org/10.4172/pharmaceutical-sciences.1000438

MOHAMMADI, R.; MOHAMMADIFAR, M. A.; MORTAZAVIAN, A. M.; ROUHI, M.; GHASEMI, J. B.; DELSHADIAN, Z. Extraction optimization of pepsin-soluble collagen from eggshell membrane by response surface methodology (RSM). **Food Chemistry,** v. 190, p. 186-193, jan. 2016. https://doi.org/10.1016/j.foodchem.2015.05.073

MOHITE, B. V.; KAMALJA, K. K.; PATIL, S. V. Statistical optimization of culture conditions for enhanced bacterial cellulose production by *Gluconoacetobacter hansenii* NCIM 2529. **Cellulose,** v. 19, n. 5, p. 1655-1666, out. 2012. https://doi.org/10.1007/s10570-012-9760-y

MOHITE, B. V.; SALUNKE, B. K.; PATIL, S. V. Enhanced production of bacterial cellulose by Using *Gluconacetobacter hansenii* NCIM 2529 strain under shaking conditions. **Applied Biochemistry and Biotechnology,** v. 169, n. 5, p. 1497-1511, mar. 2013. https://doi.org/10.1007/s12010-013-0092-7

MOURABET, M.; EL RHILASSI, A.; EL BOUJAADY, H.; BENNANI-ZIATNI, M.; TAITAI, A. Use of response surface methodology for optimization of fluoride adsorption in an aqueous solution by Brushite. **Arabian Journal of Chemistry,** v. 10, p. S3292-S3302, maio 2017. https://doi.org/10.1016/j.arabjc.2013.12.028

NUMATA, Y.; YOSHIHARA, S.; KONO, H. In situ formation and post-formation treatment of bacterial cellulose/κ-carrageenan composite pellicles. **Carbohydrate Polymer Technologies and Applications**, v. 2, p. 100059, dez. 2021. https://doi.org/10.1016/j.carpta.2021.100059

NURFARAHIN, A. H.; MOHAMED, M. S.; PHANG, L. Y. Culture medium development for microbial-derived surfactants production—an overview. **Molecules,** v. 23, n. 5, p. 1049, 2018. https://doi.org/10.3390/molecules23051049

OLIVEIRA, S. A.; SILVA, B. C.; RIEGEL-VIDOTTI, I. C.; URBANO, A.; FARIA-TISCHER, P. C. S.; TISCHER, C. A. Production and characterization of bacterial cellulose membranes with hyaluronic acid from chicken comb. **International Journal of Biological Macromolecules,** v. 97, p. 642-653, abr. 2017. https://doi.org/10.1016/j.ijbiomac.2017.01.077

PANAGOPOULOU, E. A.; CHIOU, A.; KARATHANOS, V. T. Water-soluble vitamin content of sun-dried Corinthian raisins (*Vitis vinifera L*., var. Apyrena). **Journal of the Science of Food and Agriculture,** v. 99, n. 12, p. 5327-5333, set. 2019. https://doi.org/10.1002/jsfa.9771

PAXIMADA, P.; TSOUKO, E.; KOPSAHELIS, N.; KOUTINAS, A. A.; MANDALA, I. Bacterial cellulose as stabilizer of o/w emulsions. **Food Hydrocolloids,** v. 53, p. 225-232, fev. 2016. https://doi.org/10.1016/j.foodhyd.2014.12.003

PICHETH, G. F.; PIRICH, C. L.; SIERAKOWSKI, M. R.; WOEHL, M. A.; SAKAKIBARA, C. N.; SOUZA, C. F.; MARTIN, A. A.; SILVA, R.; FREITAS, R. A. Bacterial cellulose in biomedical applications: A review. **International Journal of Biological Macromolecules,** v. 104, p. 97-106, nov. 2017. https://doi.org/10.1016/j.ijbiomac.2017.05.171

PLACKETT, R. L.; BURMAN, J. P. The design of optimum multifactorial experiments. **Biometrika,** v. 33, n. 4, p. 305-325, jun. 1946. https://doi.org/10.2307/2332195

PRASHANTH, L.; KATTAPAGARI, K. K.; CHITTURI, R. T.; BADDAM, V. R. R.; PRASAD, L. K. A review on role of essential trace elements in health and disease. **Journal of dr. NTR University of Health Sciences,** v. 4, n. 2, p. 75-85, jun. 2015. https://doi.org/10.4103/2277-8632.158577

QI, G.-X.; LUO, M.-T.; HUANG, C.; GUO, H.-J.; CHEN, X.-F.; XIONG, L.; WANG, B.; LIN, X.-Q.; PENG, F.; CHEN, X.-D. Comparison of bacterial cellulose production by *Gluconacetobacter xylinus* on bagasse acid and enzymatic hydrolysates. **Journal of Applied Polymer Science,** v. 134, n. 28, p. 45066, jul. 2017. https://doi.org/10.1002/app.45066

QIU, K.; NETRAVALI, A. N. A Review of fabrication and applications of bacterial cellulose based nanocomposites. **Polymer Reviews,** v. 54, n. 4, p. 598-626, 2014. https://doi.org/10.1080/15583724.2014.896018

QIU, X.; ZHANG, Y.; HONG, H. Classification of acetic acid bacteria and their acid resistant mechanism. **AMB Express,** v. 11, n. 1, p. 1-15, fev. 2021. https://doi.org/10.1186/s13568-021-01189-6

RAMANA, K. V.; TOMAR, A.; SINGH, L. Effect of various carbon and nitrogen sources on cellulose synthesis by *Acetobacter xylinum*. **World Journal of Microbiology and Biotechnology,** v. 16, n. 3, p. 245-248, abr. 2000. https://doi.org/10.1023/A:1008958014270

RADU, C. D.; VERESTIUC, L.; ULEA, E.; LIPSA, F. D.; VULPE, V.; MUNTEANU, C.; BULGARIU, L.; PAȘCA, S.; TAMAS, C.; CIUNTU, B. M.; CIOCAN, M.; SÎRBU, I.; GAVRILAS, E.; MACAREL, C. V.; ISTRATE, B. Evaluation of keratin/bacterial cellulose based scaffolds as potential burned wound dressing. **Applied Sciences,** v. 11, n. 5, p. 1995, 2021. https://doi.org/10.3390/app11051995

REGTI, A.; LAAMARI, M. R.; STIRIBA, S. E.; EL HADDAD, M. Use of response factorial design for process optimization of basic dye adsorption onto activated carbon derived from Persea species. **Microchemical Journal,** v. 130, p. 129-136, jan. 2017. https://doi.org/10.1016/j.microc.2016.08.012

RODRIGUES, M. I.; IEMMA, A. F. **Planejamento de experimentos e otimização de processos.** 3. ed. Campinas: Cárita, 2014.

RONGPIPI, S.; YE, D.; GOMEZ, E. D.; GOMEZ, E. W. Progress and opportunities in the characterization of cellulose–an important regulator of cell wall growth and

mechanics. **Frontiers in Plant Science**, v. 9, p. 1894, mar. 2019. https://doi.org/10.3389/fpls.2018.01894

SALARI, M.; KHIABANI, M. S.; MOKARRAM, R. R.; GHANBARZADEH, B.; KAFIL, H. S. Preparation and characterization of cellulose nanocrystals from bacterial cellulose produced in sugar beet molasses and cheese whey media. **International Journal of Biological Macromolecules,** v. 122, p. 280-288, fev. 2019. https://doi.org/10.1016/j.ijbiomac.2018.10.136

SANTOS, T. A.; SPINACÉ, M. A. S. Sandwich panel biocomposite of thermoplastic corn starch and bacterial cellulose. **International Journal of Biological Macromolecules,** v. 167, p. 358-368, jan. 2021. https://doi.org/10.1016/j.ijbiomac.2020.11.156

SANTOS JUNIOR, V. D.; NIZOLI, E.; GALVAN, D.; GOMES, R. J.; BIZ, G.; RESSUTTE, J. B.; ROCHA, T. S.; SPINOSA, W. A. Micronutrient requirements and effects on cellular growth of acetic acid bacteria involved in vinegar production. **Food Science and Technology,** 2021. https://doi.org/10.1590/fst.05121

SANTOS JUNIOR, Vitório. **Estudo das necessidades nutricionais de bactérias acéticas para a produção de ácido acético**. 2009. 86 fls. Tese (Doutorado em Ciência de Alimentos) - Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, Campinas. 2009.

SANTOSO, S. P.; CHOU, C. C.; LIN, S. P.; SOETAREDJO, F. E.; ISMADJI, S.; HSIEH, C. W.; CHENG, K. C. Enhanced production of bacterial cellulose by *Komactobacter intermedius* using statistical modeling. **Cellulose,** v. 27, n. 5, p. 2497-2509, mar. 2020. https://doi.org/10.1007/s10570-019-02961-5

SALGADO, L.; BLANK, S.; ESFAHANI, R. A. M.; STRAP, J. L.; BONETTA, D. Missense mutations in a transmembrane domain of the *Komagataeibacter xylinus* BcsA lead to changes in cellulose synthesis. **BMC Microbiology,** v. 19, p. 216, 2019. https://doi.org/10.1186/s12866-019-1577-5

SEMJONOVS, P.; RUKLISHA, M.; PAEGLE, L.; SAKA, M.; TREIMANE, R.; SKUTE, M.; ROZENBERGA, L.; VIKELE, L.; SABOVICS, M.; CLEENWERCK, I. Cellulose synthesis by *Komagataeibacter rhaeticus* strain P 1463 isolated from Kombucha. **Applied Microbiology and Biotechnology,** v. 101, n. 3, p. 1003-1012, fev. 2017. https://doi.org/10.1007/s00253-016-7761-8

SHARMILA, G.; MUTHUKUMARAN, C.; NAYAN, G.; NIDHI, B. Extracellular biopolymer production by *Aureobasidium pullulans* MTCC 2195 using jackfruit seed powder. **Journal of Polymers and the Environment,** v. 21, n. 2, p. 487-494, jun. 2013. https://doi.org/10.1007/s10924-012-0459-9

SHEYKHNAZARI, S.; TABARSA, T.; ASHORI, A.; SHAKERI, A.; GOLALIPOUR, M. Bacterial synthesized cellulose nanofibers: effects of growth times and culture mediums on the structural characteristics. **Carbohydrate Polymers,** v. 86, n. 3, p. 1187-1191, ago. 2011. https://doi.org/10.1016/j.carbpol.2011.06.011

SHI, Z.; ZHANG, Y.; PHILLIPS, G. O.; YANG, G. Utilization of bacterial cellulose in food. **Food Hydrocolloids,** v. 35, p. 539-545, mar. 2014. https://doi.org/10.1016/j.foodhyd.2013.07.012

SIEVERS, M.; SWINGS, J. Acetobacter. **Bergey's Manual of Systematics of Archaea and Bacteria,** p. 1-7, 2015. https://doi.org/10.1002/9781118960608.gbm00876

SINGH, V.; HAQUE, S.; NIWAS, R.; SRIVASTAVA, A.; PASUPULETI, M.; TRIPATHI, C. K. M. Strategies for fermentation medium optimization: an in-depth review. **Frontiers in microbiology,** v. 7, p. 2087, jan. 2017. https://doi.org/10.3389/fmicb.2016.02087

SINGH, R.; SHARMA, D. C.; GUPTA, M. K. Optimization of critical process parameters for amylase production by *Bacillus* sp. using statistical approach (RSM). **Journal of Microbiology and Biotechnology Research,** v. 7, n. 3, p. 7-15, 2017. https://doi.org/10.24896/JMBR.2017732

SINGH, A.; MANJU; YADAV, A.; BISHNOI, N. R. Statistical screening and optimization of process variables for xylanase production utilizing alkali-pretreated rice husk. **Annals of Microbiology,** v. 63, n. 1, p. 353-361, mar. 2013. https://doi.org/10.1007/s13213-012-0482-z

SON, H.-J.; KIM, H.-G.; KIM, K.-K.; KIM, H.-S.; KIM, Y.-G.; LEE, S.-J. Increased production of bacterial cellulose by *Acetobacter* sp. V6 in synthetic media under shaking culture conditions. **Bioresource Technology,** v. 86, n. 3, p. 215-219, fev. 2003. https://doi.org/10.1016/S0960-8524(02)00176-1

SOUZA, C. F. V.; VOLPATO, G.; HECK, J. X. Tecnologia do cultivo de microrganismos. In: BRUNO, A. N. **Biotecnologia II: Aplicações e Tecnologias**. Porto Alegre: Artmed, 2017.

SOUZA, E. F.; FURTADO, M. R.; CARVALHO, C. W.; FREITAS-SILVA, O.; GOTTSCHALK, L. M. Production and characterization of *Gluconacetobacter xylinus* bacterial cellulose using cashew apple juice and soybean molasses. **International Journal of Biological Macromolecules,** v. 146, p. 285-289, mar. 2020. https://doi.org/10.1016/j.ijbiomac.2019.12.180

SPEROTTO, G.; STASIAK, L. G.; GODOI, J. P. M. G.; GABIATTI, N. C.; SOUZA, S. S. A review of culture media for bacterial cellulose production: complex, chemically defined and minimal media modulations. **Cellulose,** v. 28, p. 2649-2673, fev. 2021. https://doi.org/10.1007/s10570-021-03754-5

SPINOSA, W. A.; SANTOS JÚNIOR, V. D.; GALVAN, D.; FIORIO, J. L.; GOMEZ, R. J. H. C. Vinegar rice (*Oryza sativa* L.) produced by a submerged fermentation process from alcoholic fermented rice. **Food Science and Technology,** v. 35, n. 1, p. 196-201, jan./mar. 2015. https://doi.org/10.1590/1678-457X.6605

STUMPF, T. R.; YANG, X.; ZHANG, J.; CAO, X. In situ and ex situ modifications of bacterial cellulose for applications in tissue engineering. **Materials Science and Engineering: C,** v. 82, p. 372-383, jan. 2018. https://doi.org/10.1016/j.msec.2016.11.121

TANG, S.; CHI, K.; XU, H.; YONG, Q.; YANG, J.; CATCHMARK, J. M. A covalently cross-linked hyaluronic acid/bacterial cellulose composite hydrogel for potential biological applications. **Carbohydrate Polymers,** v. 252, p. 117123, jan. 2021. https://doi.org/10.1016/j.carbpol.2020.117123

TAYEB, A. M.; TONY, M. A.; MANSOUR, S. A. Application of Box–Behnken factorial design for parameters optimization of basic dye removal using nano-hematite photo-Fenton tool. **Applied Water Science,** v. 8, n. 5, p. 1-9, ago. 2018. https://doi.org/10.1007/s13201-018-0783-x

TRIPATHY, D. B.; MISHRA, A.; CLARK, J.; FARMER, T. Synthesis, chemistry, physicochemical properties and industrial applications of amino acid surfactants: A review. **Comptes Rendus Chimie**, v. 21, n. 2, p. 112-130, fev. 2018. https://doi.org/10.1016/j.crci.2017.11.005

ULLAH, H.; SANTOS, H. A.; KHAN, T. Applications of bacterial cellulose in food, cosmetics and drug delivery. **Cellulose,** v. 23, n. 4, p. 2291-2314, ago. 2016. https://doi.org/10.1007/s10570-016-0986-y

ULLAH, H.; WAHID, F.; SANTOS, H. A.; KHAN, T. Advances in biomedical and pharmaceutical applications of functional bacterial cellulose-based nanocomposites. **Carbohydrate Polymers,** v. 150, p. 330-352, out. 2016. https://doi.org/10.1016/j.carbpol.2016.05.029

URBINA, L.; HERNÁNDEZ-ARRIAGA, A. M.; ECEIZA, A.; GABILONDO, N.; CORCUERA, M. A.; PRIETO, M. A.; RETEGI, A. By-products of the cider production: An alternative source of nutrients to produce bacterial cellulose. **Cellulose,** v. 24, n. 5, p. 2071-2082, maio 2017. https://doi.org/10.1007/s10570-017-1263-4

VALERA, M. J.; TORIJA, M. J.; MAS, A.; MATEO, E. Cellulose production and cellulose synthase gene detection in acetic acid bacteria. **Applied Microbiology and Biotechnology,** v. 99, n. 3, p. 1349-1361, fev. 2015. https://doi.org/10.1007/s00253-014-6198-1

VASCONCELOS, N. F.; FEITOSA, J. P. A.; GAMA, F. M. P.; MORAIS, J. P. S.; ANDRADE, F. K.; SOUZA FILHO, M. S. M.; ROSA, M. F. Bacterial cellulose nanocrystals produced under different hydrolysis conditions: Properties and morphological features. **Carbohydrate Polymers,** v. 155, p. 425-431, jan. 2017. https://doi.org/10.1016/j.carbpol.2016.08.090

VERMA, U.; NAIK, J. B.; PATIL, J. S.; YADAVA, S. K. Screening of process variables to enhance the solubility of famotidine with 2-HydroxyPropyl–β-Cyclodextrin & PVP K-30 by using Plackett–Burman design approach. **Materials Science and Engineering: C,** v. 77, p. 282-292, ago. 2017. https://doi.org/10.1016/j.msec.2017.03.238

WALKER, G. M.; STEWART, G. G. *Saccharomyces cerevisiae* in the production of fermented beverages. **Beverages,** v. 2, n. 4, p. 30, 2016. https://doi.org/10.3390/beverages2040030

WANG, Y. H.; FENG, J. T.; ZHANG, Q.; ZHANG, X. Optimization of fermentation condition for antibiotic production by Xenorhabdus nematophila with response surface methodology. **Journal of Applied Microbiology,** v. 104, n. 3, p. 735-744, mar. 2008. https://doi.org/10.1111/j.1365-2672.2007.03599.x

WANG, J.; TAVAKOLI, J.; TANG, Y. Bacterial cellulose production, properties and applications with different culture methods–A review. **Carbohydrate Polymers,** v. 219, p. 63-76, set. 2019. https://doi.org/10.1016/j.carbpol.2019.05.008

WANG, J.; WAN, W. Experimental design methods for fermentative hydrogen production: A review. **International Journal of Hydrogen Energy,** v. 34, n. 1, p. 235-244, jan. 2009. https://doi.org/10.1016/j.ijhydene.2008.10.008

WARDA, E. A.; ABEER, A. A. E. A.; EMAN, R. H.; MAHMOUD, A. S.; AHMED, I. Applications of Plackett–Burman and central composite design for the optimization of novel *Brevundimonas diminuta* KT277492 chitinase production, investigation of its antifungal activity. **Brazilian Archives of Biology and Technology,** v. 59, out. 2016. https://doi.org/10.1590/1678-4324-2016160245

WU, G.; BAZER, F. W.; DAI, Z.; LI, D.; WANG, J.; WU, Z. Amino acid nutrition in animals: protein synthesis and beyond. **Annual Review of Animal Biosciences,** v. 2, n. 1, p. 387-417, fev. 2014. https://doi.org/10.1146/annurev-animal-022513-114113

WU, X.; YAO, H.; CAO, L.; ZHENG, Z.; CHEN, X.; ZHANG, M.; WEI Z.; CHENG J.; JIANG S.; PAN L.; LI, X. Improving acetic acid production by over-expressing PQQ-ADH in Acetobacter pasteurianus. **Frontiers in Microbiology,** v. 8, p. 1713, set. 2017. https://doi.org/10.3389/fmicb.2017.01713

XIA, K.; HAN, C.; XU, J.; LIANG, X. Toxin-antitoxin HicAB regulates the formation of persister cells responsible for the acid stress resistance in *Acetobacter pasteurianus*. **Applied Microbiology and Biotechnology,** v. 105, n. 2, p. 725-739, jan. 2021. https://doi.org/10.1007/s00253-020-11078-w

YAMANAKA, S.; WATANABE, K.; KITAMURA, N.; IGUCHI, M.; MITSUHASHI, S.; NISHI, Y.; URYU, M. The structure and mechanical properties of sheets prepared from bacterial cellulose. **Journal of Materials Science,** v. 24, n. 9, p. 3141-3145, set. 1989. https://doi.org/10.1007/BF01139032

YANG, Q.; MA, H.; DAI, Z.; WANG, J.; DONG, S.; SHEN, J.; DONG, J. Improved thermal and mechanical properties of bacterial cellulose with the introduction of collagen. **Cellulose,** v. 24, n. 9, p. 3777-3787, set. 2017. https://doi.org/10.1007/s10570-017-1366-y

YASSINE, F.; BASSIL, N.; CHOKR, A.; SAMRANI, A. E.; SERGHEI, A.; BOITEUX, G.; TAHCHI, M. E. Two-step formation mechanism of *Acetobacter* cellulosic biofilm: Synthesis of sparse and compact cellulose. **Cellulose,** v. 23, n. 2, p. 1087-1100, abr. 2016. https://doi.org/10.1007/s10570-016-0884-3

YE, S.; JIANG, L.; SU, C.; ZHU, Z.; WEN, Y.; SHAO, W. Development of gelatin/bacterial cellulose composite sponges as potential natural wound dressings. **International Journal of Biological Macromolecules,** v. 133, p. 148-155, jul. 2019. https://doi.org/10.1016/j.ijbiomac.2019.04.095

YIM, S. M.; SONG, J. E.; KIM, H. R. Production and characterization of bacterial cellulose fabrics by nitrogen sources of tea and carbon sources of sugar. **Process Biochemistry,** v. 59, p. 26-36, ago. 2017. https://doi.org/10.1016/j.procbio.2016.07.001

YIN, H.; HE, Y.; DENG, Y.; DONG, J.; LU, J.; CHEN, L. Application of Plackett–Burman experimental design for investigating the effect of wort amino acids on flavour-active compounds production during lager yeast fermentation. **Journal of the Institute of Brewing**, v. 123, n. 3, p. 300-311, jul. 2017. https://doi.org/10.1002/jib.424

YOGANATHAN, S.; VEDERAS, J. C. Nonprotein L-Amino Acids. In: MANDER, L.; LIU, H.-W. **Comprehensive Natural Products II: Chemistry and Biology**. Elsevier, 2010. v. 5, p. 5-70.

YOLMEH, M.; JAFARI, S. M. Applications of response surface methodology in the food industry processes. **Food and Bioprocess Technology,** v. 10, n. 3, p. 413-433, jan. 2017. https://doi.org/10.1007/s11947-016-1855-2

ZEČIĆ, A.; DHONDT, I.; BRAECKMAN, B. P. The nutritional requirements of *Caenorhabditis elegans*. **Genes & nutrition,** v. 14, n. 1, p. 15, maio 2019. https://doi.org/10.1186/s12263-019-0637-7

ZENG, X.; SMALL, D. P.; WAN, W. Statistical optimization of culture conditions for bacterial cellulose production by *Acetobacter xylinum* BPR 2001 from maple syrup. **Carbohydrate Polymers,** v. 85, n. 3, p. 506-513, jun. 2011. https://doi.org/10.1016/j.carbpol.2011.02.034

ZHANG, H.; XU, X.; CHEN, X.; YUAN, F.; SUN, B.; XU, Y.; YANG, J.; SUN, D. Complete genome sequence of the cellulose-producing strain *Komagataeibacter nataicola* RZS01. **Scientific Reports,** v. 7, n. 1, p. 1-8, jun. 2017. https://doi.org/10.1038/s41598-017-04589-6

ZHANG, Z. Y.; SUN, Y.; ZHENG, Y. D.; HE, W.; YANG, Y. Y.; XIE, Y. J.; FENG, Z. X.; QIAO, K. A biocompatible bacterial cellulose/tannic acid composite with antibacterial and anti-biofilm activities for biomedical applications. **Materials Science and Engineering: C,** v. 106, p. 110249, jan. 2020. https://doi.org/10.1016/j.msec.2019.110249

ZHAO, B.; JIANG, H.; LIN, Z.; XU, S.; XIE, J.; ZHANG, A. Preparation of acrylamide/acrylic acid cellulose hydrogels for the adsorption of heavy metal ions. **Carbohydrate Polymers,** v. 224, p. 115022, nov. 2019. https://doi.org/10.1016/j.carbpol.2019.115022

ZHOU, L. L.; SUN, D. P.; HU, L. Y.; LI, Y. W.; YANG, J. Z. Effect of addition of sodium alginate on bacterial cellulose production by *Acetobacter xylinum*. **Journal of Industrial Microbiology & Biotechnology,** v. 34, p. 483-483, jul. 2007. https://doi.org/10.1007/s10295-007-0218-4

ZHOU, P.; WANG, J.; WANG, M.; HOU, J.; LU, J. R.; XU, H. Amino acid conformations control the morphological and chiral features of the self-assembled peptide nanostructures: Young investigators perspective. **Journal of Colloid and Interface Science**, v. 548, p. 244-254, jul. 2019. https://doi.org/10.1016/j.jcis.2019.04.019

CAPÍTULO II – MICRONUTRIENTS IRON, ZINC, MANGANESE, PANTOTHENATE AND NICOTINIC ACID PLAY AN IMPORTANT ROLE IN BACTERIAL CELLULOSE PRODUCTION BY ACETIC ACID BACTERIA

ABSTRACT

As other living organisms, microorganisms require sources of chemical elements and nutrients, such as minerals and vitamins, which are essential for their multiplication and metabolite production. So, the knowledge of the nutritional needs of microorganisms that have important industrial applications is necessary for the nutrients to be supplied in the appropriate form and amount. Acetic acid bacteria (AAB) comprise a variety of microorganisms that are used to obtain many products. Among these products, bacterial cellulose (BC) is a biopolymer with high purity and crystallinity that has several applications in the medical, pharmaceutical and food industry. Therefore, this study aimed to evaluate the nutritional requirements of two AAB strains (*Komagataeibacter hansenii* ATCC 23769 and *Komagataeibacter intermedius* V-05) in qualitative and quantitative level aiming to increase the production and properties of BC by the strains. Several micronutrients (five minerals and ten vitamins) were tested, selected and optimized through a Plackett & Burman factorial design and central composite design to determine the best concentration of each required nutrient. Besides that, membranes produced at optimal conditions by each strain were characterized in relation to chemical structure and properties. As result, five micronutrients were required for one or both strains. Among them, *K. hansenii* required iron (20 mg L^{-1}), zinc (5 mg L^{-1}), pantothenic acid (20 mg L^{-1}) and nicotinic acid (7 mg L^{-1}), while *K. intermedius* required iron (15 mg L^{-1}), manganese (15 mg L^{-1}), pantothenic acid (6 mg L^{-1}) and nicotinic acid (6 mg L^{-1}). After optimization and validation steps, a production level of 1.35 g L^{-1} and 2.23 g L^{-1} was achieved by *K. hansenii* and *K. intermedius*, respectively. Membranes produced from optimized media by the strain *K. hansenii* present greater yield, crystallinity and thermal properties than those produced from standard HS medium, while the production in optimized media by the strain *K. intermedius* improved thermal and hydrophilic properties compared to those from HS medium. Through this work, it was observed that different strains of the same genus may have different nutritional requirements. The supply of the correct nutrients in their most appropriate concentrations favored the microbial development and formation of products and may increase BC production or properties compared to non-optimized media.

Key words: fermentation; microbial nutrition; minerals; vitamins; biopolymer.

4.2.1. Introduction

The group of microorganisms commonly known as “acetic acid bacteria” (AAB) are mainly involved in the production of vinegar due to their high ability to synthesize acetic acid from ethanol in the presence of oxygen. Furthermore, some species of the genera *Gluconacetobacter* and *Komagataeibacter* are also able to synthesize very important microbial exopolysaccharides, such as levan and bacterial cellulose (BC) (CHINA et al., 2018; VALERA et al., 2015; QIU; ZHANG; HONG, 2021).

BC is a linear homopolymer that consists only of repeated D-glucose units $(C_6H_{10}O_5)_n$, alternately rotated 180º and connected through β-(1,4)-glycosidic bonds (GIBSON, 2012; ZHAO et al., 2019). This biopolymer is identical to plant cellulose in relation to its chemical composition and molecular structure. However, BC presents superior physicochemical properties including high purity and crystallinity, ultrafine reticulated network, good thermal and mechanical properties, excellent water-holding capacity and good biodegradability and biocompatibility. In addition, BC is free of impurities, such as lignin, hemicellulose and other vegetal cell wall compounds, consequently eliminating the need to use severe chemical treatment for removing them. BC from culture medium contains only microbial cells, nutrients and other secondary metabolites that can be removed through a mild alkaline treatment, thus obtaining a high-purity cellulose (BLANCO PARTE et al., 2020; CHENG et al., 2017; LOTFIMAN et al., 2018; VASCONCELOS et al., 2017).

The formulation of an ideal medium involves the selection of right nutrients at their correct levels to provide an ideal microenvironment that can promote microbial growth and metabolite production (NURFARAHIN; MOHAMED; PHANG, 2018). In fermentation processes that involve a large number of variables, the two-level fractional factorial design of Plackett & Burman (PLACKETT; BURMAN, 1946) has been widely used to identify critical variables that can be selected or eliminated in further optimization processes. In this method, the exact quantity and interactions between variables are not considered. However, it provides some important informations about each factor by relatively few experiments (HOSNY et al., 2018; GÜNDOĞDU et al., 2016; SINGH et al., 2013; VERMA et al., 2017). The response surface methodology (RSM) refers to a collection of experimental strategies and statistical inference used to construct a functional relationship between a response variable and a set of dependent variables. By using RSM, the relationship among the

variables can be expressed in the form of a mathematical model which is used to plot contour curves and determine the optimal conditions of the variables for the highest response of interest (BERINGHS et al., 2015; HAMMAMI et al., 2020; YOLMEH; JAFARI, 2017). In biotechnological processes, the application of experimental designs based on statistical techniques can result in better product yields and closer confirmation of the interest response. Various researchers have applied the optimization through factorial design and RSM analysis to optimize process parameters, including temperature, pH, aeration and medium components (AJE; ADIE, 2020; BOUDRAHEM; ZIANI; AISSANI-BENISSAD, 2019).

Fermentative production is considered as a transformation of culture medium nutrients into microbial biomass and diverse metabolic products. Thus, it is necessary to provide all the nutrients required by the microorganisms for this transformation. Because nutritional needs of microorganisms may vary from strain to strain, the knowledge of microbial nutrition is necessary for the nutrients to be supplied in the suitable form and amount (MANDENIUS; BRUNDIN, 2008; NURFARAHIN; MOHAMED; PHANG, 2018). The importance of macronutrients for BC production, such as carbon, nitrogen, calcium, magnesium and vitamin C has been shown in several works (JAGANNATH et al., 2008; KESHK, 2014; MOHITE; KAMALJA; PATIL, 2012). However, no reports were found regarding statistical optimization of micronutrients and growth factors required for the biosynthesis of BC by acetic acid bacteria. These micronutrients comprise several metal ions and growth factors that are required in small amounts because at higher concentration they are potentially toxic (KOUR et al., 2019).

The growth factors include essential organic compounds that are not synthesized by organisms, so they need to be directly obtained from the environment. Among them, vitamins are the main ones, especially the B-complex vitamins. Vitamins are considered organic growth factors because they are essential for the metabolism of all living organisms and play an important role in microbial growth. However, in some bacteria there are no enzymes needed for the biosynthesis of certain vitamins. In this case, required vitamins need to be added to the culture medium. The most of these vitamins act mainly as coenzymes, which are non-protein components of enzymes (ALTERTHUMM, 2020; MADIGAN et al., 2016; YAO et al., 2018; TORTORA et al., 2017).

Based on this, the aim of this work was to evaluate the effects of medium supplementation with minerals (boron, iron, manganese, molybdenum and zinc) and B-complex vitamins (biotin, cyanocobalamin, pantothenic acid, folic acid, *myo*-inositol, niacin, *p*-aminobenzoic acid, pyridoxine, riboflavin and thiamine) on bacterial cellulose production by *Komagataeibacter hansenii* and *Komagataeibacter intermedius* and to characterize the samples produced by each strain in relation to chemical structure, thermal and hydrophilic properties.

4.2.2. Material and Methods

4.2.2.1. Material

Two strains belonging to the genus *Komagataeibacter* were used in this study. *Komagataeibacter hansenii* ATCC 23769 was obtained from collection culture (Embrapa Tropical Agroindustry, Brazil) and *Komagataeibacter intermedius* V-05 was previously isolated from vinegar industry (GOMES et al., 2021).

B-complex vitamins (biotin, calcium pantothenate, cyanocobalamin, folic acid, nicotinic acid, *p*-aminobenzoic acid, pyridoxine hydrochloride, riboflavin and thiamine hydrochloride) and *myo*-inositol used for experimental investigations were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cellulase enzyme (Celumax C®) was purchased from Prozyn (São Paulo, SP, Brazil). All others chemical reagents were of analytical grade and purchased from different sources: Himedia (Mumbai, India), Anidrol (Diadema, SP, Brazil), Inlab (Diadema, SP, Brazil), Nuclear (Diadema, SP, Brazil), Synth (Diadema, SP, Brazil) and Vetec Química (Rio de Janeiro, RJ, Brazil).

4.2.2.2. Activation of microorganisms

Bacterial colonies previously grown on mannitol–yeast extract–peptone (MYP) agar (25 g L^{-1} mannitol, 5 g L^{-1} yeast extract, 3 g L^{-1} peptone and 10 g L^{-1} agar), were cultivated in tubes containing 10 mL HS medium, composed of 20 g L^{-1} glucose, 5 g L^{-1} peptone, 5 g L^{-1} yeast extract, 2.7 g L^{-1} Na_2HPO_4 and 1.15 g L^{-1} citric acid (HESTRIN; SCHRAMM, 1954). After growth under static conditions (30 ± 0.5 °C), the cultivated broth was transferred to new flasks containing 100 mL HS medium, which

was used as inoculum after incubation period (30 °C for 5 days) in microbiological incubator (Tecnal TE-391) under static conditions. Subsequently, hydrolysis of the BC formed on the surface of each medium was carried out using 0.02% cellulase enzyme (1-2 days at 30 ± 0.5 °C).

4.2.2.3. Basal medium, inoculum and culture conditions

For analyzing the effects on BC production by adding micronutrients, a yeast extract and peptone-free culture medium was used for following supplementation with selected minerals and vitamins. For this, it was elaborated a synthetic medium, containing 50 g L^{-1} of carbon source (sucrose) and 10 g L^{-1} of nitrogen source ($(NH_4)_2SO_4$) (YAMANAKA et al., 1989). The basal medium also contained 2 g L^{-1} of sodium and phosphate ions source (NaH_2PO_4) and 1 g L^{-1} of magnesium ion source ($MgSO_4.7H_2O$). The pH of the medium was adjusted at 6.00 ± 0.10 with 1 M KOH. Finally, 10 mL L^{-1} ethanol was added as an additional energy source (LI et al., 2012; MOHITE et al., 2012; NARITOMI et al., 1998). After hydrolysis of the inoculum (section 4.2.2.2.), the cells were centrifuged (Eppendorf Centrifuge 5804R, Germany) for 15 min, at 5000 rpm (5 °C), for removing culture medium interferers. A cell suspension containing 10^8 colony-forming units (CFU) per mL was transferred to flaks containing basal medium (100 mL) supplemented with varying concentrations of minerals and vitamins, according to the experiments of screening and optimization.

4.2.2.4. Prepare of micronutrients solution

According to literature data, Ten B-complex vitamins and five minerals were used to screen the most important factors required for improvement of bacterial cellulose production by the strains. Vitamins used were: biotin (B_7), pantothenic acid (calcium pantothenate, B_5), cyanocobalamin (B_{12}), folic acid (B_9), *myo*-inositol, niacin (nicotinic acid, B_3), *p*-aminobenzoic acid (PABA), pyridoxine (pyridoxine hydrochloride, B_6), riboflavin (B_2) and thiamine (thiamine hydrochloride, B_1). Minerals used were: boron (H_3BO_3), iron ($FeSO_4.7H_2O$), molybdenum ($(NH_4)_6Mo_7O_{24}.4H_2O$), zinc ($ZnSO_4.7H_2O$) and manganese ($MnSO_4.H_2O$) (SANTOS JUNIOR et al., 2021; HEO; SON, 2002; MATSUOKA et al., 1996; RUKA; SIMON; DEAN, 2012). Sterile

solutions containing exactly 1 g L^{-1} of each vitamin were prepared by solubilization in Milli-Q® ultrapure water (Merck Millipore, Billerica, MA, USA), followed by filtration in 0.22 µm polyvinylidene fluoride (PVDF) sterile syringe filter (Durapore®, Millipore Millex - GV, Ireland). Solutions containing exactly 1 g L^{-1} of each mineral were prepared by using the molar mass of the elements B, Fe, Mo, Zn and Mn. These minerals and vitamins solutions of known concentration were used in the experiments of screening and optimization.

4.2.2.5. Experimental design for selection of micronutrients

Initially, it was made the screening of the most significant micronutrients that affect bacterial cellulose production by the strains using the Plackett & Burman design. A total of fifteen micronutrients were tested to evaluate their effects on the response of interest. The actual values of the variables are given in Table 1 where each factor is represented at two levels: high concentration (+1) and low concentration (−1). The experimental design consisted of 20 runs where each column contained equal number of positive and negative signs (Table A2). Four trials at the central point (0) were added to design in order to verify the repeatability of the process. The experimental design was generated and analyzed by using statistical software R® (R Core Team, Version 3.6.3). Response variables were the mass of BC produced by each strain (BC_{KH} and BC_{KI} for *K. hansenii* and *K. intermedius*, respectively) expressed in dry weight (g L^{-1}). The effect of each variable was equal to the difference between the average of the measurements made at the high concentration level of the factor and the average of the measurements made at the low concentration level of the same factor. The resultant effect of each variable was determined by the following equation (DU et al., 2021):

$$E(\mathbf{X}i) = 2\,\frac{(\sum \mathbf{M}i^{+} - \sum \mathbf{M}i^{-})}{N}$$

where $E(\mathbf{X}i)$ is the concentration effect of the tested variable; $\mathbf{M}i^{+}$ and $\mathbf{M}i^{-}$ represent BC production from the runs where the measured variables $\mathbf{X}i$ were present at high and low concentrations, respectively; and N is the total number of runs that was

equal to 20. The dry weight of BC produced in g L^{-1} was considered as the response. The significant level (*p*-value) of each variable effect was determined using Student's t-test (SINGH et al., 2017):

$$t(\mathbf{X}i) = \frac{E(\mathbf{X}i)}{\mathbf{SE}}$$

where $E(\mathbf{X}i)$ is the effect of variable $\mathbf{X}i$ and **SE** is the standard error of the concentration effect that was equal to square root of the variance of an effect. Variables with confidence level greater than 90% were considered to influence the BC production significantly.

4.2.2.6. Central composite design

It was applied the central composite design (CCD) in order to find out the optimum levels of the most effective variables identified from the screening step results, check the effects of each variable and check the interaction effects between them. Thus, a 2^4 factorial design with eight axial points and four replicates at the central point was employed using different combinations of the variables, totaling 28 randomized trials. The coded and actual values of the four variables used in the experiments (X_1, X_2, X_3 and X_4) at five fixed levels (-α, -1, 0, +1 and +α) are given in Tables 2 and 3. Response variables were the mass of BC produced by each strain (BC_{KH} and BC_{KI} for *K. hansenii* and *K. intermedius*, respectively) expressed in dry weight (g L^{-1}). By using the statistical software R® (R Core Team, Version 3.6.3), it was generated and analyzed the experimental design as well as determined the estimated effects, regression coefficients, analysis of variance (ANOVA) and the optimal conditions of the essential micronutrients.

The second-order polynomial model was represented by the following quadratic equation (ZHANG et al., 2017):

$$\mathrm{Y} = \beta_0 + \sum \beta_i \mathrm{X}_i + \sum \beta_{ii} \mathrm{X}_i^2 + \sum \beta_{ij} \mathrm{X}_i \mathrm{X}_j$$

where: $\mathbf{Y}$ is the predicted response; $\boldsymbol{\beta_0}$ is the intercept term; $\boldsymbol{\beta_i}$ is the linear coefficient; $\boldsymbol{\beta_{ii}}$ is the squared coefficient; $\boldsymbol{\beta_{ij}}$ is the interaction coefficient; and $\mathbf{X_i}$ is the coded value of the independent variables.

Models were validated using Student's t-test (α = 0.05), where the average of the experimental values (n = 5) were compared with the predicted values of BC. Another experiment was carried out in HS medium to compare the BC production in the optimized synthetic medium and in a standard complex medium (HS). These samples were dried at 60 ºC to be characterized as described in section 4.2.2.8.

4.2.2.7. Quantification of bacterial cellulose produced

After 10 days, the bacterial cellulose produced on the surface of each experimental medium were collected and heated in 2% (w/v) NaOH solution for 1 h at 80 ºC and then, washed with distilled water until neutral pH (NÚÑEZ et al., 2020). After alkaline treatment, the membranes were dried until obtaining constant weight to determine the respective yields expressed in dry weight (g L^{-1}).

4.2.2.8. Characterization of bacterial cellulose produced

4.2.2.8.1. Fourier transform infrared spectroscopy (FT-IR)

Infrared spectra were recorded using a spectrometer (Shimadzu, Prestige-21, Japan) over the 4000–400 cm^{-1} range. The scans were taken for dried BC samples mixed with potassium bromide (KBr). The spectra were collected in transmission mode, with a resolution of 1 cm^{-1}.

4.2.2.8.2. X-ray diffraction (XRD)

XRD patterns of the dried BC samples were obtained using a X-ray diffractometer (Malvern Panalytical X'Pert PRO MPD, Almelo, Netherlands) in the Bragg-Brentano geometry with Cu-Kα radiation (wavelength λ = 1.5418 Å) at 2θ range from 5 to 70º and angular step of 0.04º. The radiation source voltage and

electric current were 40 kV and 40 mA, respectively. Counting time per point was 3 s. The crystallinity index was determined for cellulose I as follows (SEGAL et al., 1959):

$$\textbf{Crystallinity Index (\%)} = \frac{(I_{002} - Iam)}{I_{002}} \times 100$$

where I_{002} is the maximum peak intensity corresponding to the 002 lattice diffraction at angle 2θ = 22.8°; and **Iam** is the intensity of diffraction corresponding to the amorphous background at angle 2θ = 18°.

4.2.2.8.3. Thermogravimetric analysis (TGA)

Thermal analysis of BC samples (approximately 5 mg) was performed using a thermal analyser (Shimadzu TGA-50, Tokyo, Japan). The scans were ramped from room temperature to 600 ºC at a heating rate of 10 ºC min^{-1}, under nitrogen atmosphere (50 mL min^{-1}). Derivative form of TG curves (DTG) was obtained using differential of TGA values. The TGA and DTG curves were expressed as the mass variation as a function of temperature.

4.2.2.8.4. Differential scanning calorimetry (DSC)

DSC curves were obtained using a differential scanning calorimeter (Shimadzu DSC-60, Tokyo, Japan). The scans were started at room temperature and completed at 400 ºC under a nitrogen atmosphere (50 mL min^{-1}) and heating rate of 10 ºC min^{-1}. The instrument was calibrated with indium standard.

4.2.2.8.5. Hydrophilic properties

For determination of the water holding capacity (WHC), rehydration capacity and moisture content, never-dried samples were shaken quickly and weighed after being removed from the storage recipient (BC_{wet}). Then, the samples were dried until the water was completely removed, and weighed again (BC_{dry}).

WHC was calculated by the following formula (FENG et al., 2015):

$$\textbf{Water holding capacity} = \frac{(BC_{wet} - BC_{dry})}{BC_{dry}}$$

The dried membranes (BC_{dry}) were immersed in distilled water until the weight of the rehydrated sample (BC_r) no longer increased. The reabsorption capacity was calculated by the following formula (TEIXEIRA et al., 2019):

$$\textbf{Rehydration capacity (\%)} = \frac{(BC_r - BC_{dry})}{BC_r} \textbf{ x 100}$$

The moisture content of the BC was obtained by using the following formula (CHANDRASEKARAN; BARI; SINHA, 2017):

$$\textbf{Moisture content (\%)} = \frac{(BC_{wet} - BC_{dry})}{BC_{wet}} \textbf{ x 100}$$

4.2.3. Results and Discussion

4.2.3.1. Effect analysis of micronutrients on the bacterial cellulose production by acetic acid bacteria

Table 1 shows the experimental plan involving 15 independent variables and corresponding mass of BC synthetized by the strains in each one of the 24 experimental runs. In this assay, BC_{KH} varied from 0.36 to 1.10 g L^{-1} and BC_{KI} varied from 0.88 to 2.19 g L^{-1}. Based on the results, the effect of each variable was calculated and the significance level of the variables was determined (Table A3). The coefficient of determination (R^2) were equal to 0.8728 for BC_{KH} and 0.9186 for BC_{KI}, which means that 87.28% and 91.86% of the data variability can be explained by the model, respectively. It was observed that required nutrients varied between the strains. On the one hand, for *K. hansenii* 23769 obtained from collection culture, the

Table 1. Experimental design of Plackett & Burman containing the actual values of the independent variables and response variables of bacterial cellulose produced by the strains *K. hansenii* ATCC 23769 **(BC_{kH})** and *K. intermedius* V-05 **(BC_{KI})**.

Run	Independent variables															Response variables	
	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}	BC_{KH}	BC_{KI}
1	0.50	0	5.0	3.0	0	0	0	0	5.0	0	10.0	0	3.0	1.0	3.0	0.36	1.48
2	0.50	5.0	0	3.0	3.0	0	0	0	0	5.0	0	3.0	0	1.0	3.0	0.86	1.60
3	0	5.0	5.0	0	3.0	5.0	0	0	0	0	10.0	0	3.0	0	3.0	0.71	1.59
4	0	0	5.0	3.0	0	5.0	3.0	0	0	0	0	3.0	0	1.0	0	0.76	1.90
5	0.50	0	0	3.0	3.0	0	3.0	1.0	0	0	0	0	3.0	0	3.0	0.91	1.72
6	0.50	5.0	0	0	3.0	5.0	0	1.0	5.0	0	0	0	0	1.0	0	0.85	1.47
7	0.50	5.0	5.0	0	0	5.0	3.0	0	5.0	5.0	0	0	0	0	3.0	0.84	1.93
8	0.50	5.0	5.0	3.0	0	0	3.0	1.0	0	5.0	10.0	0	0	0	0	0.92	2.19
9	0	5.0	5.0	3.0	3.0	0	0	1.0	5.0	0	10.0	3.0	0	0	0	1.10	1.87
10	0.50	0	5.0	3.0	3.0	5.0	0	0	5.0	5.0	0	3.0	3.0	0	0	0.93	1.53
11	0	5.0	0	3.0	3.0	5.0	3.0	0	0	5.0	10.0	0	3.0	1.0	0	1.02	2.12
12	0.50	0	5.0	0	3.0	5.0	3.0	1.0	0	0	10.0	3.0	0	1.0	3.0	0.87	1.75
13	0	5.0	0	3.0	0	5.0	3.0	1.0	5.0	0	0	3.0	3.0	0	3.0	0.81	1.91
14	0	0	5.0	0	3.0	0	3.0	1.0	5.0	5.0	0	0	3.0	1.0	0	0.70	1.79
15	0	0	0	3.0	0	5.0	0	1.0	5.0	5.0	10.0	0	0	1.0	3.0	0.45	0.88
16	0	0	0	0	3.0	0	3.0	0	5.0	5.0	10.0	3.0	0	0	3.0	0.86	1.98
17	0.50	0	0	0	0	5.0	0	1.0	0	5.0	10.0	3.0	3.0	0	0	0.82	1.41
18	0.50	5.0	0	0	0	0	3.0	0	5.0	0	10.0	3.0	3.0	1.0	0	0.89	1.89
19	0	5.0	5.0	0	0	0	0	1.0	0	5.0	0	3.0	3.0	1.0	3.0	0.79	1.61
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.67	1.32
21	0.25	2.5	2.5	1.5	1.5	2.5	1.5	0.5	2.5	2.5	5.0	1.5	1.5	0.5	1.5	0.72	1.80
22	0.25	2.5	2.5	1.5	1.5	2.5	1.5	0.5	2.5	2.5	5.0	1.5	1.5	0.5	1.5	0.70	1.83
23	0.25	2.5	2.5	1.5	1.5	2.5	1.5	0.5	2.5	2.5	5.0	1.5	1.5	0.5	1.5	0.86	1.72
24	0.25	2.5	2.5	1.5	1.5	2.5	1.5	0.5	2.5	2.5	5.0	1.5	1.5	0.5	1.5	0.89	1.76

Legend: X_1 – boron (mg L^{-1}); X_2 – iron (mg L^{-1}); X_3 – manganese (mg L^{-1}); X_4 – molybdenum (mg L^{-1}); X_5 – zinc (mg L^{-1}); X_6 – folic acid (µg L^{-1}); X_7 – pantothenate (mg L^{-1}); X_8 – *p*-aminobenzoic acid (mg L^{-1}); X_9 – biotin (µg L^{-1}); X_{10} – cyanocobalamin (mg L^{-1}); X_{11} – inositol (mg L^{-1}); X_{12} – niacin (mg L^{-1}); X_{13} – pyridoxine (mg L^{-1}); X_{14} – riboflavin (mg L^{-1}); X_{15} – thiamine(mg L^{-1}).

nutrients that showed more significant positive effect were: zinc ($p < 0.01$), iron ($p = 0.01$), niacin ($p < 0.05$) and pantothenate ($p < 0.05$). A significant negative effect was observed for riboflavin ($p < 0.05$) and thiamine ($p < 0.05$). On the other hand, for *K. intermedius* V-05 isolated from vinegar industry, the nutrients that showed more significant positive effect were: pantothenate ($p < 0.001$), iron ($p < 0.005$) and manganese ($p < 0.1$). No significant negative effect was observed for the micronutrients tested by this strain.

The micronutrients that present more significant and positive effects were selected to determine their optimal levels by using response surface methodology, *i.e*. iron, zinc, pantothenate and niacin, for *K. hansenii* and iron, manganese and pantothenate for *K. intermedius*. Niacin had a slightly positive effect for *K. intermedius* and was also selected to optimize its response by this strain. Because the lowest concentration level (-1) was equal 0 mg L^{-1} for all tested nutrients, the factors that had a significant negative effect were removed from the optimization experiments. Remaining components, *i.e*. boron, molybdenum, folic acid, *p*-aminobenzoic acid, biotin, cyanocobalamin, *myo*-inositol and pyridoxine showed low confidence level ($p > 0.1$) and were considered insignificant in this study.

Among minerals and B-complex vitamins tested for BC biosynthesis, all the 4-period elements (Fe^{2+}, Mn^{2+} and Zn^{2+}), and vitamins pantothenic acid and niacin were required for one or both strains. Among the metal ions required by both strains, iron is an important nutrient for the growth and survival of the most bacterial species and is essential to nearly all known organisms. Iron is a trace element that plays an important role in bacterial physiology as an essential component of metabolic enzymes and regulatory proteins (FRAWLEY; FANG, 2014).

As it is a d-block transition metal, iron can interchange between various oxidation states, which allow it to participate in redox reactions and also bind to several biological ligands. In cellular respiration, iron is a key component of enzymes that are involved in electron transfer reactions, namely cytochromes and iron–sulfur (Fe-S) proteins (YIANNIKOURIDES; LATUNDE-DADA, 2019; ROUT; SAHOO, 2015). Cytochromes are respiratory chain proteins that are oxidized and reduced through loss or gain of electron by the iron element contained in the “heme”, the iron-containing portion of the cytochromes.

The heme group can be alternated between the reduced form (Fe^{2+}) and the oxidized form (Fe^{3+}) through the oxidation reaction (MADIGAN et al., 2016):

$$\text{cytochrome–}Fe^{2+} \leftrightarrow \text{cytochrome–}Fe^{3+} - e^{-}$$

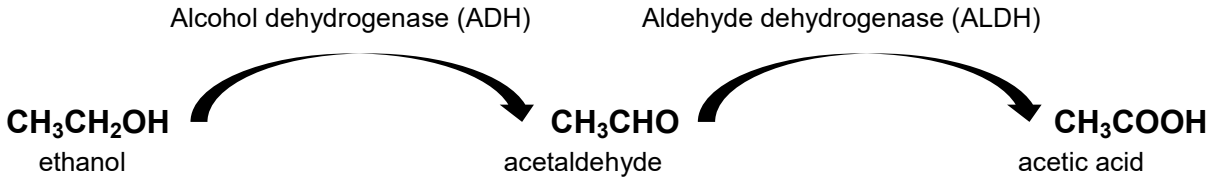
Cytochromes also mediate the production of high-energy phosphorous compounds, such as adenosine triphosphate (ATP) and uridine triphosphate (UTP). It is reported that these compounds contribute to bacterial polysaccharide biosynthesis like BC in AAB strains, although this mechanism is still unknown (ALMEIDA et al., 2013; SON et al., 2003). Iron was required for both strains while the other inorganic divalent cations, *i.e*. zinc and manganese were required for either *K. intermedius* (manganese requirement) or *K. hansenii* (zinc requirement).

Manganese is an essential transition element present in low concentrations in most living organisms due to its important biological functions. This metal exists in various oxidation states but the Mn^{2+} oxidation state is the most prevalent in biological systems. Manganese is a co-factor for approximately 6% of all known metalloenzymes. The main one is the superoxide dismutase (SOD), an antioxidant enzyme that plays a critical role against oxidative stress caused by reactive oxygen species (ROS). AAB are aerobic organisms that utilize molecular oxygen (O_2) as final electron receptor in the oxidative phosphorylation and electron transport chain. Generally, O_2 is reduced to H_2O, however, partial reduction of O_2 leads to the formation of ROS, including the superoxide anion (O_2^-), the hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH\cdot$). ROS are strong oxidizing agents that can oxidize macromolecules and organic compounds in the cell causing damage to biomolecules as DNA and RNA, protein oxidation and lipid peroxidation. Therefore, all aerobic organisms possess an anti-oxidative defense system that includes SOD and catalase enzymes, responsible for eliminating superoxide and hydrogen peroxide, respectively. The dismutation mechanism involves the alternation between the oxidised (Mn^{3+}) and less oxidised (Mn^{2+}) state of Mn. The electron derived from superoxide oxidation is transferred to Mn^{3+}, producing Mn^{2+}-SOD and O_2 (ISMY et al., 2020; LI; ZHOU, 2011; MADIGAN et al., 2016; SCHMIDT; HUSTED, 2019; SHEDID et al., 2018).

Zinc is another essential element for the growth and development of all organisms. It is a d-block metal taken up as divalent cation Zn^{2+}, which possesses a stable oxidation state, eliminating the probability of redox reactions as in iron and manganese. Many proteins have zinc as cofactor. This trace element is involved in

cellular growth and others multiple cellular functions, such as enzymatic reactions, DNA synthesis, gene expression, stabilization of proteins and as component of several metalloenzymes including DNA and RNA polymerases and alkaline phosphatase (KANDARI; JOSHI; BHATNAGAR, 2021; SHAO et al., 2014; CHASAPIS et al., 2020). Zinc can also function as an activator of the enzyme alcohol dehydrogenase (ADH). This enzyme is present in AAB and is of fundamental importance in the bioconversion of ethanol to acetic acid because it catalyzes the oxidation of ethanol to acetaldehyde (Figure 1). Due to its important biological functions, many bacteria exhibit zinc uptake-dependency as observed in this study (MONDAL; BOSE, 2019; GOMES et al., 2018; KANDARI et al., 2019).

Figure 1. Sequential enzymatic reactions for converting ethanol into acetic acid.



Both required vitamins are found to be precursors of intracellular coenzymes that are necessary to regulate vital biochemical reactions in the cell. Pantothenic acid (also known as vitamin B_5 or, when ionized, as pantothenate) is an essential micronutrient that stimulate the growth of a wide range of organisms, from bacteria to plants and animals. Its name is derived from the Greek word *pantothen*, meaning “from everywhere” due to its occurrence in both plant and animal foods. Pantothenic acid is a key precursor for the biosynthesis of coenzyme A (CoA). This cofactor is involved in many metabolic reactions, including the synthesis of phospholipids, synthesis and degradation of fatty acids, and the operation of the tricarboxylic acid cycle (TCA), which is one of the stages of cellular respiration to produce energy from carbohydrate catabolism. Living cells synthesize CoA from pantothenic acid by a series of enzymatic steps starting with the phosphorylation of pantothenate to 4’-phosphopantothenate catalyzed by pantothenate kinase (LEONARDI; JACKOWSKI, 2007; SHEDID et al., 2018; SPRY; KIRK; SALIBA, 2008; TORTORA et al., 2017).

The term niacin (also known as vitamin B_3) includes nicotinic acid (or pyridine-3-carboxylic acid) and its amide, nicotinamide (or pyridine-3-carboxamide). Both compounds are nutritional precursors of essential coenzymes in cellular metabolism: nicotinamide adenine dinucleotide (NAD^+), its phosphorylated form nicotinamide adenine dinucleotide phosphate ($NADP^+$) and their respective reduced forms (NAD(P)H). NAD and NADP are essential to cellular metabolism and respiration as they participate as electron carriers in a large number of redox reactions. NAD can be synthesized from either B_3-vitamin group members or some amino acids. In the Preiss–Handler pathway, nicotinic acid is converted to NAD^+ through three metabolic conversions by the action of nicotinic acid phosphoribosyltransferase, nicotinamide mononucleotide adenylyltransferase and NAD synthase enzymes, respectively. Moreover, addition of a phosphate group to the adenosine ribose of NAD^+ by NAD kinase leads to a formation of $NADP^+$ (BRAIDY et al., 2019; KATSYUBA; AUWERX, 2017; KIRKLAND; MEYER-FICCA, 2018; MAKAROV; TRAMMELL; MIGAUD, 2019; SRIVASTAVA, 2016; VANLINDEN; SKOGE; ZIEGLER, 2015).

NAD(P)H cofactors are essential for oxidative processes and implicated in many anabolic and catabolic pathways in all forms of life. While the redox pair NAD^+/NADH is mainly used by enzymes that catalyze oxidative and catabolic reaction, $NADP^+$/NADPH are mainly used by enzymes that catalyze reductive and anabolic reactions. For example, NAD is involved in the glycolysis, TCA cycle and oxidative phosphorylation leading to energy production in the cell. NADP, on the other hand, plays a key role in cellular defense against oxidative stress, as well as in the synthesis of fatty acids and DNA (KIRKLAND; MEYER-FICCA, 2018; MAKAROV; TRAMMELL; MIGAUD, 2019; BRAIDY et al., 2019; KATSYUBA; AUWERX, 2017; VANLINDEN; SKOGE; ZIEGLER, 2015; YING, 2008). Many dehydrogenases use these molecules to serve as electron acceptors or donors. AAB partially oxidize ethanol through two successive catalytic reactions catalyzed by the ADH and a membrane-bound aldehyde dehydrogenase (ALDH) (Figure 1). These PQQ-dependent enzymes are bound to the periplasmic side and involved only in extracellular acetic acid production. However, AAB also have additional ADH and ALDH, within the cytoplasm at lower levels, which are NAD-dependent dehydrogenases. NAD-dependent ADHs are not involved in acetic acid production, but they are principally involved in complete oxidation of ethanol through TCA cycles (CHINNAWIROTPISAN et al., 2003; MAMLOUK; GULLO, 2013).

Two variables had significant negative effect in this study: riboflavin and thiamine, mainly for *K. hansenii*. Riboflavin (also known as vitamin B_2) function as an important precursor of coenzymes *i.e.*, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). These cofactors are mostly involved in redox reactions of cells and participate in the metabolism of carbohydrates, lipids, and proteins from which living organisms obtain their energy (AVERIANOVA et al., 2020). Thiamine (also known as vitamin B_1) after phosphorylation to thiamine diphosphate acts as a coenzyme of many enzymes catalyzing various carboxylation and decarboxylation reactions (TYLICKI et al., 2018).

Several species of bacteria and fungi, including the AAB strain *Gluconobacter oxydans* are able to synthesize riboflavin through the riboflavin biosynthetic pathway (RBP), which starts from guanosine-5′-triphosphate and ribulose-5-phosphate (AVERIANOVA et al., 2020; CISTERNAS; SALAZAR; GARCÍA-ANGULO, 2018; NOMAN et al., 2020). Thiamine-producing microorganisms, such as *Escherichia coli* and lactic acid bacteria have been also reported (LEONARDI; ROACH, 2004; TERAN et al., 2021). This ability could eliminate the need for exogenous supply of B_1 and B_2 vitamins in the culture medium. However, synthesis of thiamine and riboflavin by *Komagataeibacter* species has not been reported yet.

A study about the effects on BC production by *K. sucrofermentans* BPR2001 adding individually one of 12 tested vitamins showed that nicotinic acid, *p*-aminobenzoic acid, biotin and pyridoxine were the most stimulating vitamins in a synthetic medium containing fructose as carbon source. Riboflavin had the most negative effect showing to decrease the BC productivity (ISHIKAWA et al., 1995). In another report about the effects of several nutrients on BC production carried out by Son et al. (2003), it was observed that the individual addition of iron, boron and niacin increased the level of production by an *Acetobacter* sp. V6 in a synthetic medium containing glucose as carbon source. Keshk (2014) also verified that the presence of vitamin C in HS medium increased the yield of BC in all strains of *Gluconacetobacter* spp. tested. The investigation of the nutritional requirements of cultures of *A. melanogenum*, *A. oxydans* and *A. rancens* showed that these species mainly required pantothenic acid, *p*-aminobenzoic acid, and nicotinic acid in a synthetic medium containing glucose as carbon source.

Table 2. Coded and actual values of the independent variables analyzed in the central composite design.

Variable	Variable Code	Levels (mg L^{-1})*				
		-α	-1	0	+1	+α
***K. hansenii* 23769**						
Iron	X_1	0.00	5.00	10.0	15.0	20.0
Zinc	X_2	0.00	3.00	6.00	9.00	12.0
Pantothenate	X_3	0.00	5.00	10.0	15.0	20.0
Niacin	X_4	0.00	3.00	6.00	9.00	12.0
***K. intermedius* V-05**						
Iron	X_1	0.00	5.00	10.0	15.0	20.0
Manganese	X_2	0.00	5.00	10.0	15.0	20.0
Pantothenate	X_3	0.00	3.00	6.00	9.00	12.0
Niacin	X_4	0.00	3.00	6.00	9.00	12.0

*The levels '-1', '+1' and '0' represent respectively the low, high and medium concentration of the variables. Symbol 'α' represents the axial point (α = 2.0).

4.2.3.2. Analysis of central composite design

Table 3 shows the central composite design involving four independent variables and response variables observed in each one of the 28 experimental runs. In this assay BC_{KH} varied from 0.33 to 1.44 g L^{-1} and BC_{KI} varied from 1.33 to 2.35 g L^{-1} dry weight. Tables A4 and A5 present the estimated effects and analysis of variance (ANOVA) on the results of BC produced by both strains, respectively. For BC_{KH}, it was observed that coefficient values of X_1 and X_3 were positive and linear and quadratic effects were very significant ($p < 0.05$), which indicated that increasing the level of iron and pantothenate positively affected the production of bacterial cellulose. Moreover, the interaction coefficient of X_1 and X_3 had significant positive effect (p = 0.001) and it was higher than the isolated effects of the variables. This result indicated that there was strong interaction among these parameters that positively influenced the BC production by *K. hansenii* 23769. The coefficient of determination (R^2 = 0.8380) was satisfactory to confirm the significance of the model indicating that 83.80% of the data variability can be explained by the predicted

model. The lack-of-fit was not significant (p > 0.10) indicating that the predicted model was well-adjusted to the data.

Table 3. Central composite experimental design containing the coded values of the independent variables and response variables of bacterial cellulose produced by the strains *K. hansenii* ATCC 23769 (**BC_{kH}**) and *K. intermedius* V-05 (**BC_{KI}**).

Run	Independent variables (mg L^{-1})				Response variables (g L^{-1})	
	X_1	X_2	X_3	X_4	BC_{KH}	BC_{KI}
1	-1	-1	-1	-1	0.71	1.49
2	-1	-1	-1	1	0.67	1.55
3	-1	-1	1	-1	0.88	1.33
4	-1	-1	1	1	0.79	1.39
5	-1	1	-1	-1	0.80	1.94
6	-1	1	-1	1	0.73	1.87
7	-1	1	1	-1	0.53	1.58
8	-1	1	1	1	0.55	1.63
9	1	-1	-1	-1	0.71	1.68
10	1	-1	-1	1	0.89	1.57
11	1	-1	1	-1	1.39	1.37
12	1	-1	1	1	1.44	1.46
13	1	1	-1	-1	0.47	2.32
14	1	1	-1	1	0.33	2.35
15	1	1	1	-1	1.04	1.93
16	1	1	1	1	1.18	1.91
17	-2	0	0	0	0.59	1.32
18	2	0	0	0	0.99	2.06
19	0	-2	0	0	0.69	1.56
20	0	2	0	0	0.92	2.04
21	0	0	-2	0	0.48	1.49
22	0	0	2	0	0.69	1.59
23	0	0	0	-2	0.85	1.89
24	0	0	0	2	1.03	2.08
25	0	0	0	0	1.01	2.23
26	0	0	0	0	1.13	2.09
27	0	0	0	0	1.23	2.16
28	0	0	0	0	1.12	2.24

Legend: **X_1** – iron; **X_2** – zinc (for *K. hansenii*) or manganese (for *K. intermedius*); **X_3** – pantothenate; **X_4** – niacin.

For BC_{KI}, it was observed that coefficient values of X_1 and X_2 were positive and the linear and quadratic effects were very significant ($p < 0.005$), which indicated that increasing the level of iron and manganese positively affected the production of bacterial cellulose. Moreover, the interaction effect of X_1 and X_2 was slightly significant ($p < 0.10$) and positive indicating that there was an interaction among these parameters that influenced the BC production by *K. intermedius* V-05. The coefficient of determination ($R^2 = 0.9028$) was satisfactory to confirm the significance of the model indicating that 90.28% of the data variability can be explained by the predicted model. The lack-of-fit was not significant ($p > 0.05$) indicating that the predicted model was well-adjusted to the data.

From the results achieved in the central composite design, it was obtained a second-order regression model that explains the dependence of BC production on the nutrients of culture medium and provides the levels of BC as a function of the concentration of iron, zinc, pantothenate and niacin, by *K. hansenii* ATCC 23769, and iron, manganese, pantothenate and niacin, by *K. intermedius* V-05. The levels of BC produced by each strain can be predicted by the following quadratic polynomial equation in which terms in bold are significant at the 90% confidence level:

$$\mathbf{Y_{K.\ hansenii}} = \mathbf{1.12\ (\pm\ 0.08) + 0.108}x_1\ \mathbf{(\pm\ 0.033)} - 0.058x_2\ (\pm\ 0.033) + \mathbf{0.121}x_3\ \mathbf{(\pm\ 0.033)} + 0.017x_4\ (\pm\ 0.033) - \mathbf{0.077}x_1^2\ \mathbf{(\pm\ 0.033) - 0.073}x_2^2\ \mathbf{(\pm\ 0.033) - 0.128}x_3^2\ \mathbf{(\pm\ 0.033)} - 0.039x_4^2\ (\pm\ 0.033) - 0.060x_1x_2\ (\pm\ 0.041) + \mathbf{0.176}x_1x_3\ \mathbf{(\pm\ 0.041)} + 0.026x_1x_4\ (\pm\ 0.041) - 0.034x_2x_3\ (\pm\ 0.041) - 0.009x_2x_4\ (\pm\ 0.041) + 0.012x_3x_4\ (\pm\ 0.041) \quad \textbf{Eq. (1)}$$

$$\mathbf{Y_{K.\ intermedius}} = \mathbf{2.18\ (\pm\ 0.07) + 0.137}x_1\ \mathbf{(\pm\ 0.030) + 0.194}x_2\ \mathbf{(\pm\ 0.030) - 0.082}x_3\ \mathbf{(\pm\ 0.030)} + 0.020x_4\ (\pm\ 0.030) - \mathbf{0.130}x_1^2\ \mathbf{(\pm\ 0.030) - 0.102}x_2^2\ \mathbf{(\pm\ 0.030) - 0.167}x_3^2\ \mathbf{(\pm\ 0.030) - 0.056}x_4^2\ \mathbf{(\pm\ 0.030) + 0.073}x_1x_2\ \mathbf{(\pm\ 0.036)} - 0.021x_1x_3\ (\pm\ 0.036) - 0.007x_1x_4\ (\pm\ 0.036) - 0.043x_2x_3\ (\pm\ 0.036) - 0.007x_2x_4\ (\pm\ 0.036) + 0.017x_3x_4\ (\pm\ 0.036) \quad \textbf{Eq. (2)}$$

where $\mathbf{Y}$ is the response variable (BC production), x_1 is the coded value of variable X_1 (iron), x_2 is the coded value of variable X_2 (zinc for *K. hansenii* and manganese for *K. intermedius*), x_3 is the coded value of variable X_3 (pantothenate) and x_4 is the coded value of variable X_4 (niacin). The observed and predicted values determined by Equations (1) and (2) are shown in Figures A3 and A4, respectively.

Figure 2 present the contour surface plots showing dependency of BC_{KH} on the variables studied at different concentration levels. As observed in Figures 2(a), 2(b) and 2(c), the increase in iron concentration resulted in significant increase in mass of BC produced by *K. hansenii* 23769.

Similarly, the increase in pantothenate concentration also showed to increase the mass of BC_{KH}, at the same level as observed with iron addition (Figures 2(b), 2(d) and 2(f)). From the quadratic polynomial Equation (1) and the response surface plots, the optimal region of iron and pantothenate were found to be closer to the positive axial point (+2), *i.e.*, 20.0 mg L^{-1}.

The increase in zinc concentration resulted in slight and gradual decrease of BC_{KH} (Figures 2(a), 2(d) and 2(e)) and the increase or decrease in nicotinic acid concentration showed not significantly to increase or decrease the mass of BC_{KH} (Figures 2(c), 2(e) and 2(f)). From the quadratic polynomial Equation (1) and the response surface plots, the optimal regions of zinc and nicotinic acid were found to be closer to the central point (0), *i.e.*, 5.00 and 7.00 mg L^{-1}, respectively.

Figure 3 represent the contour surface plots showing the dependency of BC_{KI} on the variables studied at different concentration levels. As observed for BC_{KH}, the increase in iron concentration resulted in significant increase in mass of BC_{KI} (Figures 3(a), 3(b) and 3(c)). From the quadratic polynomial Equation (2) and the response surface plots, the optimal region of iron was found to be closer to the (+1) level, *i.e.*, 15.0 mg L^{-1}.

The increase in manganese concentration showed to increase BC production, at the same level as observed with iron addition (Figure 3(a), 3(d) and 3(e)). From the quadratic polynomial Equation (2) and the response surface plots, the optimal region of manganese was found to be closer to the (+1) level, *i.e.*, 15.0 mg L^{-1}.

The increase in pantothenate concentration resulted in slight and gradual decrease of BC_{KI} (Figures 3(b), 3(d) and 3(f)). From the quadratic polynomial Equation (1) and the response surface plots, the optimal regions of pantothenic acid was found to be closer to the central point (0), *i.e.*, 6.00 mg L^{-1}. Finally, the increase or decrease in nicotinic acid concentration showed not significant to increase or decrease the mass of BC_{KI} (Figures 3(c), 3(e) and 3(f)). From the quadratic polynomial Equation (2) and the response surface plots, the optimal regions of this nutrient was found to be closer to the central point (0), *i.e.*, 6.00 mg L^{-1}.

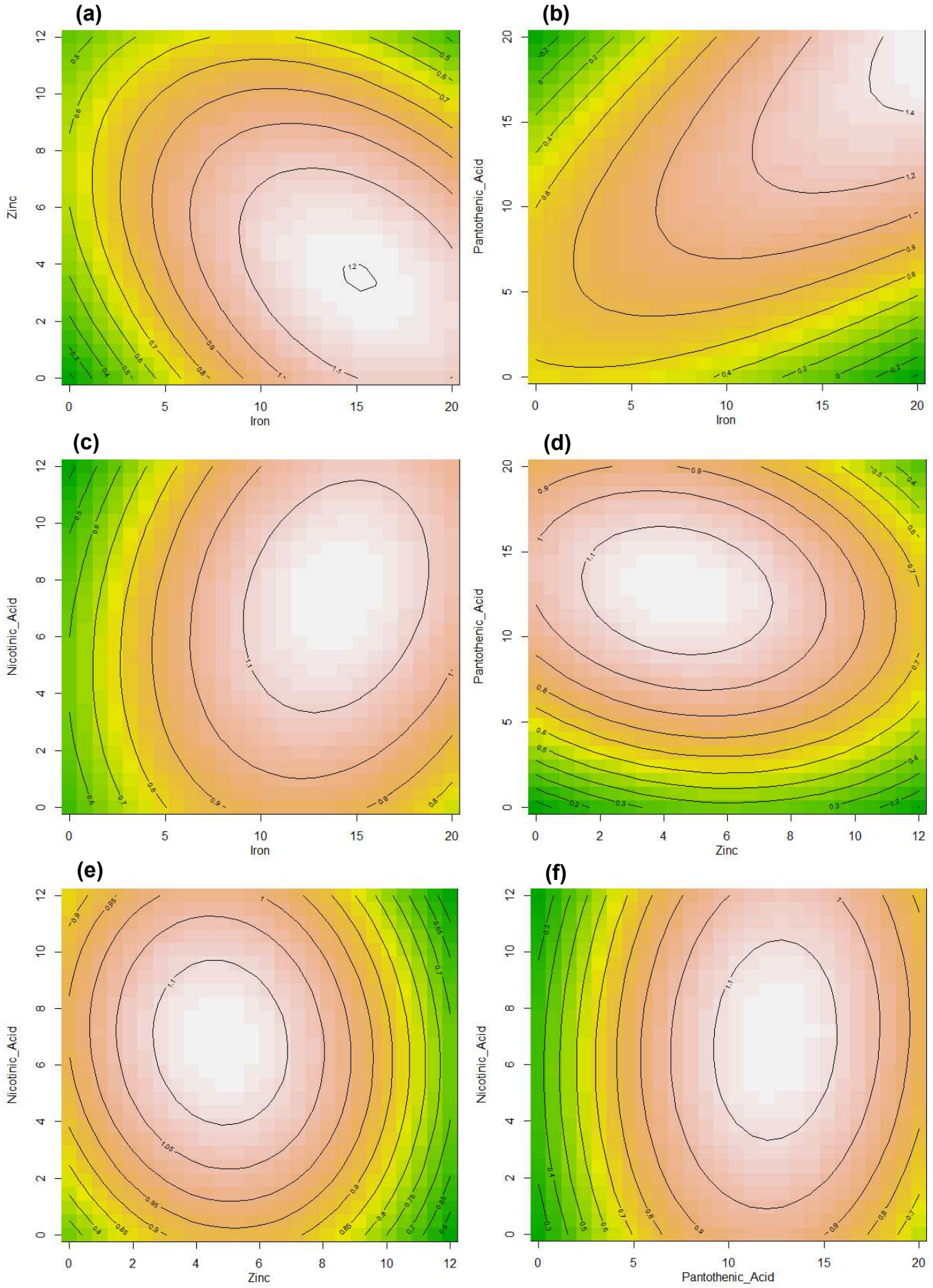


Figure 2. Contour curves for the BC production as a function of concentration (mg L^{-1}) of iron and zinc **(a)**, iron and pantothenic acid **(b)**, iron and nicotinic acid **(c)**, zinc and pantothenic acid **(d)**, zinc and nicotinic acid **(e)**, and pantothenic acid and nicotinic acid **(f)** required by strain *K. hansenii* ATCC 23769.

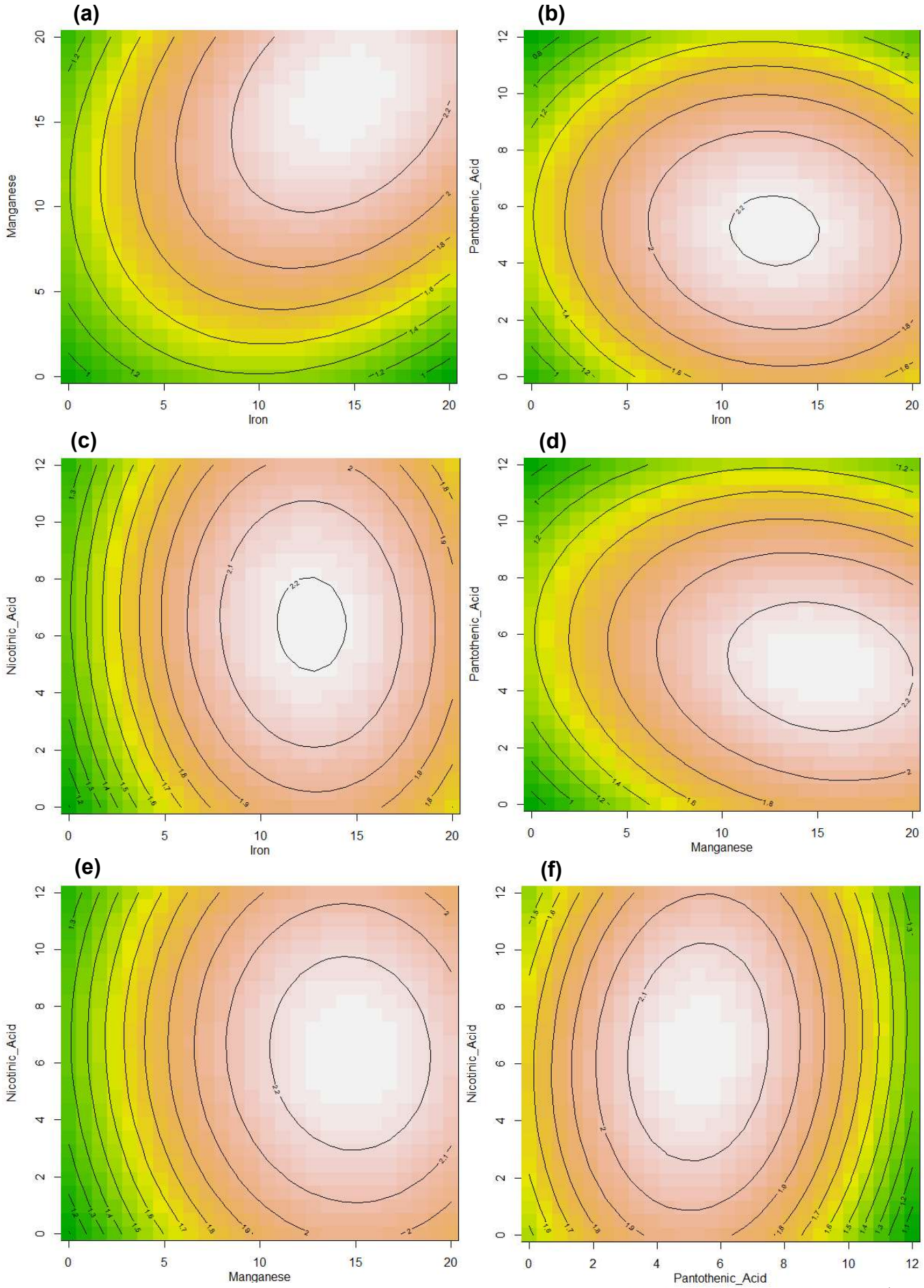


Figure 3. Contour curves for the BC production as a function of concentration (mg L^{-1}) of iron and manganese **(a)**, iron and pantothenic acid **(b)**, iron and nicotinic acid **(c)**, manganese and pantothenic acid **(d)**, manganese and nicotinic acid **(e)**, and pantothenic acid and nicotinic acid **(f)** required by strain *K. intermedius* V-05.

4.2.3.3. Validation of the experimental model

Verification of the calculated optimal conditions for BC production was done by performing the experiment at predicted optimized conditions for each strain. For BC_{KH}, the optimal concentrations estimated by the response surface model were: 20.0 mg L^{-1} iron, 20.0 mg L^{-1} pantothenate, 5.00 mg L^{-1} zinc and 7.00 mg L^{-1} niacin. For BC_{KI}, the optimal concentrations estimated by the response surface model were: 15.0 mg L^{-1} iron, 15.0 mg L^{-1} manganese, 6.00 mg L^{-1} pantothenate and 6.00 mg L^{-1} niacin. Table 4 shows the results of validation. The maximum predicted BC production that can be obtained using the optimum concentrations of variables were: 1.48 g L^{-1} and 2.35 g L^{-1}, for BC_{KH} and BC_{KI}, respectively.

The observed values for BC production were very close ($p < 0.05$) to the values estimated by the predictive models. For that reason, these models can be used for predictive purposes. The strains differed in the mass of BC produced in both synthetic and HS-complex medium, the most used medium for BC production. On the one hand, the strain *K. hansenii* 23759 produced more BC in optimized synthetic medium (1.35 g L^{-1}) than in the complex medium (0.61 g L^{-1}). On the other hand, BC production by the strain *K. intermedius* was higher in complex medium (3.43 g L^{-1}) than in optimized synthetic medium (2.23 g L^{-1}). This difference may be related to the carbon and nitrogen source used in the media. In the optimized medium, sucrose was used as carbon source and $(NH_4)_2SO_4$ was used as nitrogen source. Conversely, in the standard HS medium, glucose was used as carbon source and yeast extract and peptone were used as nitrogen source.

Table 4. Results of validation obtained using the quadratic model for optimal concentration of variables that affect bacterial cellulose production by the strains *K. hansenii* ATCC 23769 and *K. intermedius* V-05.

Strain	Bacterial cellulose (g L^{-1})		
	Predicted values	**Observed values**	**Standard medium (HS)**
***K. hansenii* ATCC 23769**	1.48	1.35 ± 0.12	0.61 ± 0.07
***K. intermedius* V-05**	2.35	2.23 ± 0.15	3.43 ± 0.19

Difference in BC production yield according to the carbon source has been observed by other authors and it may occur by varying the strain and the carbon source in the media. As example, cellulose production was higher with glucose instead of sucrose as a carbon source by the strains *K. medellinensis* (MOLINA-RAMÍREZ et al., 2017) and *K. xylinus* PTCC 1734 (TABAII; EMTIAZI, 2016). Conversely, cellulose production was higher with sucrose instead of glucose as carbon source by the strains *K. sucrofermentans* CECT 7291 (SANTOS; CARBAJO; VILLAR, 2013) and *K. xylinus* ATCC 53524 (MIKKELSEN et al., 2009). According to Rao and Stokes (1953), an appropriate medium must contain not only required growth factors but also appropriate sources of carbon and energy.

From the mass of each inorganic element required, *i.e*. iron, manganese and zinc, it was possible calculate the weight of the corresponded compounds, namely $FeSO_4.7H_2O$, $MnSO_4.H_2O$ and $ZnSO_4.7H_2O$, equivalent to the mass required in the optimized synthetic medium (Table 5). These corresponded compounds are the most sources of the required metal ions used in culture media for microbiological process. Thus, the amount of iron (20.0 mg L^{-1}) and zinc (5.00 mg L^{-1}) required by the strain *K. hansenii* is corresponding to approximately 99.3 mg L^{-1} $FeSO_4.7H_2O$ and 22.0 mg L^{-1} $ZnSO_4.7H_2O$. Similarly, the amount of iron (15 mg L^{-1}) and manganese (15.0 mg L^{-1}) required by the strain *K. intermedius* is corresponding to approximately 74.5 mg L^{-1} $FeSO_4.7H_2O$ and 46.0 mg L^{-1} $MnSO_4.H_2O$.

Table 5. Mass conversion of required metal ions by the strains *K. hansenii* ATCC 23769 and K*. intermedius* V-05 for equivalent weight of iron, manganese and zinc compounds sources.

Element	Molecular weight (g mol^{-1})	Amount required (mg L^{-1})	Equivalent compound source	Equivalent compound weight (mg L^{-1})*
Iron (Fe)	56.0	17.5 (average)	$FeSO_4.7H_2O$	87.0
Manganese (Mn)	55.0	15.0	$MnSO_4.H_2O$	46.0
Zinc (Zn)	65.0	6.00	$ZnSO_4.7H_2O$	22.0

*Considering the molecular weight of the compounds: $FeSO_4.7H_2O$ (278.0 g mol^{-1}); $MnSO_4.H_2O$ (169.0 g mol^{-1}); $ZnSO_4.7H_2O$ (287.5 g mol^{-1}).

4.2.3.4. FT-IR analysis

Determination of functional groups of BC samples produced in standard (HS) and optimized media by the strains was made analysing the spectra obtained by FT-IR spectroscopy. As shown in Figure 4, the optimized synthetic media did not change the profile of BC spectra compared to the standard complex media.

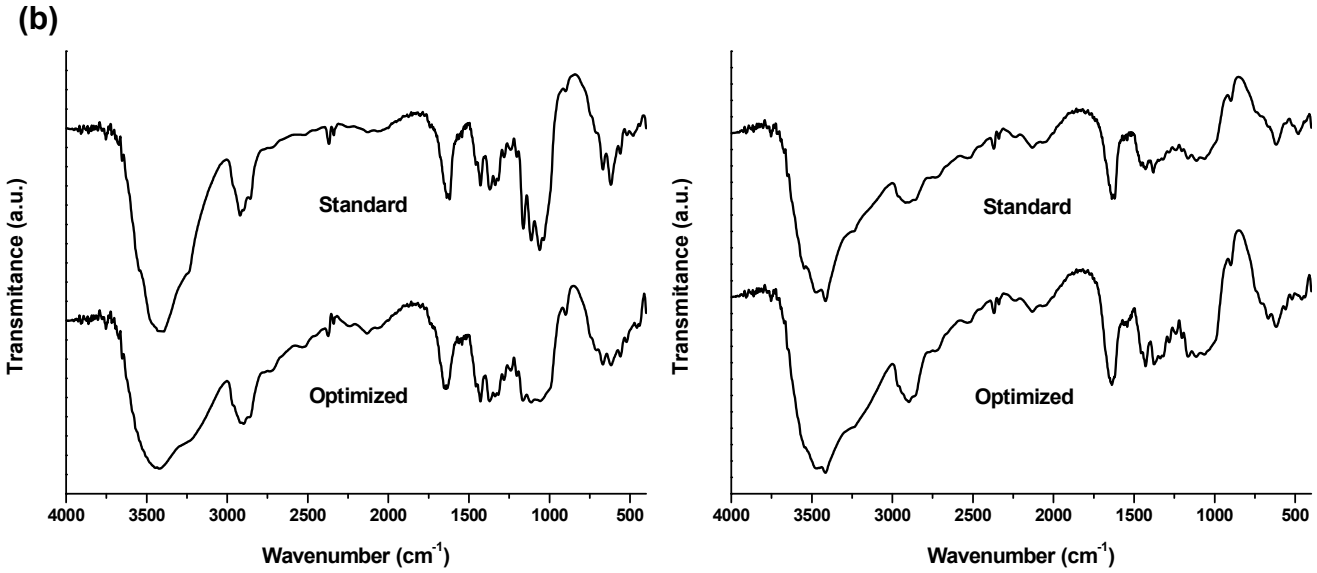
The analyzed samples presented the main bands that characterize cellulose biopolymer (Table 6), such as: strong transmission of O-H stretching vibrations at 3400-3500 cm^{-1}; alkane C-H stretching and CH_2 asymmetric stretching vibration at 2900 cm^{-1}; CH_2 symmetric stretching vibration at 2700 cm^{-1}; O-H deformation vibration at 1600 cm^{-1}; CH_2 deformation vibration at 1400 cm^{-1}; CH_3 deformation vibration at 1370 cm^{-1}; O-H deformation vibration at 1340 cm^{-1}; and C-O deformation vibration in the range of 1320-1030 cm^{-1} (KAČURÁKOVÁ et al., 2002; KONDO 1997; SUGIYAMA et al., 1991). Absence of bands at 1700-1735 cm^{-1} associated with functional groups present in proteins (C=O) and other bands of nitrogenous organic compounds mainly at 1538 cm^{-1} indicated that the purification process was efficient.

Table 6. Band assignments in the FT-IR spectra of cellulose membranes produced by AAB strains *K. hansenii* ATCC 23769 **(KH)** and *K. intermedius* V-05 **(KI)** in standard **(ST)** and optimized **(OP)** media.

Band assignments	Wave number (cm^{-1})			
	KH-ST	**KH-OP**	**KI-ST**	**KI-OP**
O-H stretching vibration	3412	3422	3416	3416
C-H stretching vibration	2920	2899	2918	2897
CH_2 stretching vibration	2737	2737	2735	2735
O-H deformation vibration	1620	n.d.	1620	n.d.
CH_2 deformation vibration	1427	1427	1429	1429
CH_3 deformation vibration	1371	1371	1381	1377
C-O deformation vibration	1113	1113	1113	1113
H-O-H bending vibration of absorbed water	1638	1638	1638	1638
Contribution from celulose Iα	n.d.	n.d.	n.d.	n.d.
Contribution from celulose Iβ	n.d.	708	n.d.	706

n.d. = not detected.

Figure 4. FT-IR spectra of the bacterial cellulose produced by AAB strains *K. hansenii* ATCC 23769 **(a)** and *K. intermedius* V-05 **(b)** in standard and optimized medium.



Bands observed at 1640 cm^{-1} (H-O-H) and 3500 cm^{-1} (O-H) were attributed to water absorption by the composites and the bands observed around 740 cm^{-1} and 710 cm^{-1} correspond respectively to phases Iα and Iβ of the BC samples (POGORELOVA et al., 2020). The results of FT-IR analysis are in agreement with studies carried out previously (POGORELOVA et al., 2020) indicating that the substances produced were chemically pure bacterial cellulose.

4.2.3.5. TG and DTG analysis

Thermal decomposition and the respective DTG curves of the membranes are shown in Figure 5. Membranes produced in optimized medium presented typical and similar profiles compared to when HS medium was used. TG curves of the samples indicated three distinct mass-loss steps, which are characteristics of pure BC. The first mass-loss event was observed from room temperature (approximately 30 ºC) to approximately 150 ºC, and was attributed to mass loss due to evaporation of residual water resulting from the drying process. The second mass-loss event was observed over the temperature range from 250 to 400 ºC and was attributed to degradation of cellulose (dehydration and decomposition of the glycosidic units). The third and final event which extended up to 600 ºC and corresponded to the thermo-oxidative

breakdown (pyrolysis) of carbonaceous residues, resulting in gaseous products of low molecular weight such as carbon monoxide (VASCONCELOS et al., 2017; SALVI et al., 2014). Souza et al. (2020) have reported a similar thermal stability and degradation temperature of bacterial cellulose as observed in this work.

Figure 5. TGA **(a)** and DTG **(b)** curves of bacterial cellulose membranes produced by AAB strains *K. hansenii* ATCC 23769 **(KH)** and *K. intermedius* V-05 **(KI)** in standard **(ST)** and optimized **(OP)** media.

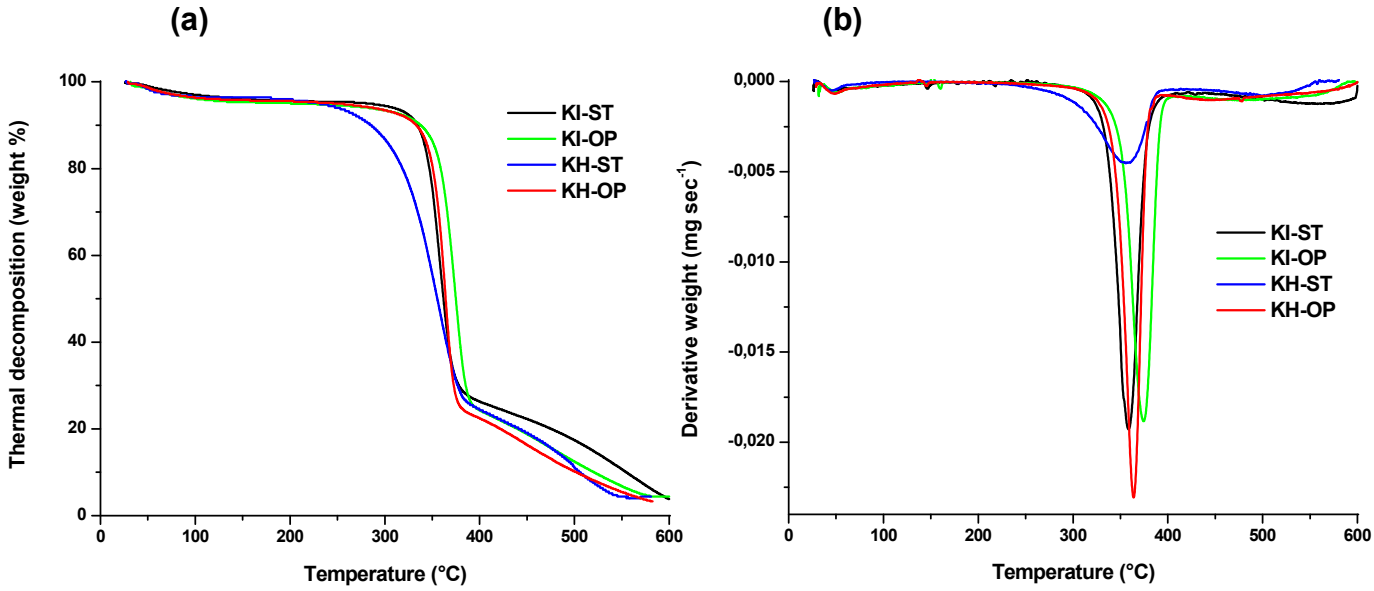


Table 7. TGA and XRD parameters of cellulose membranes produced by AAB strains *K. hansenii* ATCC 23769 **(KH)** and *K. intermedius* V-05 **(KI)** in standard **(ST)** and optimized **(OP)** media.

Sample	Thermal Decomposition					X-Ray Diffraction
	T_{onset}[a] (°C)	T_{endset}[b] (°C)	T_{max}[c] (°C)	$T_{10\%}$[d] (°C)	Weight loss (%)	CrI (%)[e]
KH-ST	290.8	380.2	355.9	286.3	95.6	60.7
KH-OP	330.7	378.3	363.9	333.0	96.7	83.5
KI-ST	332.5	375.0	358.5	334.5	96.2	91.0
KI-OP	335.9	389.5	374.3	338.3	95.6	82.5

[a] = temperature of initial thermal decomposition; [b] = temperature of final thermal decomposition; [c] = temperature of maximum weight loss rate; [d] = temperature where 10% mass was lost; [e] = crystallinity index.

From the plotted curves were determined the parameters (temperatures) of: initial thermal decomposition (T_{onset}), final thermal decomposition (T_{endset}), 10% mass loss ($T_{10\%}$), maximum weight loss rate (T_{max}) and mass loss at 600 °C. BC produced in optimized medium exhibited the highest temperatures at beginning and finishing of thermal degradation (T_{onset} = 335.9 ºC and T_{endset} = 389.5 ºC) (Table 7). It was observed that the production in optimized medium also slightly increased the temperature of maximum weight loss compared to the production in HS medium. These facts demonstrate that membranes obtained from optimized medium required higher energy for their degradation. The maximum weight loss rates were around 355-360 ºC and 360-375 ºC for membranes produced in HS and optimized medium, respectively, similar to the reported in other studies (353.5-370 ºC) (LIN et al., 2016; MOLINA-RAMÍREZ et al., 2020; SURMA-ŚLUSARSKA; PRESLER; DANIELEWICZ, 2008). Table 8 also confirms that the maximum weight loss occurred between 300 and 400 ºC showing that the samples are stable up to this temperature.

Table 8. Weight loss by temperature range of cellulose membranes produced by AAB strains *K. hansenii* ATCC 23769 **(KH)** and *K. intermedius* V-05 **(KI)** in standard **(ST)** and optimized **(OP)** media.

Temperature range	Weight loss (%)			
	KH-ST	**KH-OP**	**KI-ST**	**KI-OP**
30-100°C	3.6	3.7	3.0	3.8
100-200°C	0.4	0.7	1.4	1.3
200-300°C	9.2	2.2	1.2	1.5
300-400°C	62.5	71.1	68.3	69.2
400-500°C	13.1	12.2	8.8	11.8
500-600°C	6.8	6.8	13.5	8.0

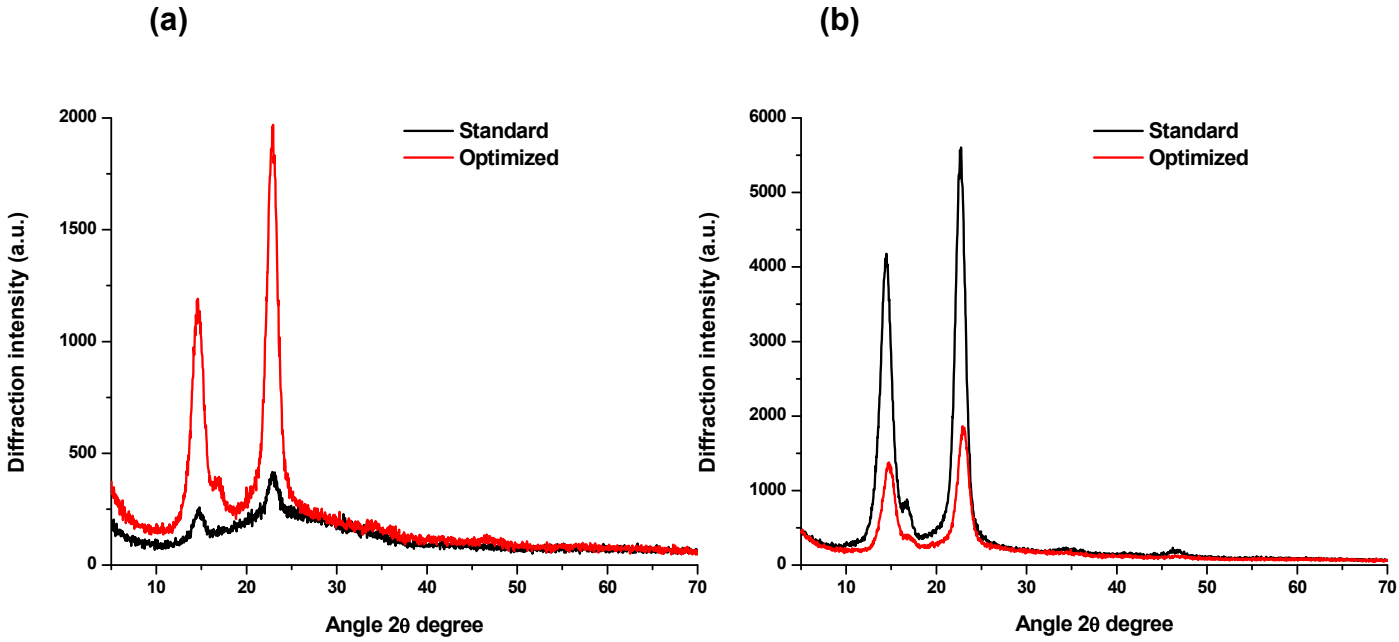
4.2.3.6. XRD analysis

The XRD data demonstrated that the culture medium did not significantly influence the crystal organization of the BC membranes, since they showed similar crystalline profiles (Figure 6). The two main peaks observed at 14.8º and 22.5º demonstrate that the samples possess typical crystalline forms of cellulose I.

Table 4 presents the crystallinity index of the BC membranes calculated from the related peak intensity. The membranes obtained from optimized medium by the strain *K. hansenii* showed crystallinity index slightly higher (83.5%) than those from HS medium (60.7%). However, the same improvement was not observed by the strain *K. intermedius*. This fact may be related to the lower yield achieved by this strain in the optimized medium compared to HS.

The values of crystallinity index observed in this work varied between 60.7% to 91.0% depending on the strain and media used. These results were similar to the reported by other authors in previous studies of BC produced from overripe banana (82.9%; MOLINA-RAMÍREZ et al., 2020), cheese whey (61.9%; SALARI et al., 2019), water waste of candied jujube (64.9-80.9%; LI et al., 2015) and wine distillery (83.0%; WU; LIU, 2013), after acid hydrolysis (90.3%; MARTÍNEZ-SANZ; LOPEZ-RUBIO; LAGARON, 2011), and in presence of ascorbic acid (62.0-86.7%; KESHK, 2014). The high crystallinity index of the samples produced by *K. hansenii* ATCC 23769 and *K. intermedius* V-05 in optimized media may indicates an increase of crystalline regions and hydrophobic interactions by these samples, making them interesting sources for being used to stabilize water/oil (W/O) emulsions (LI et al., 2018).

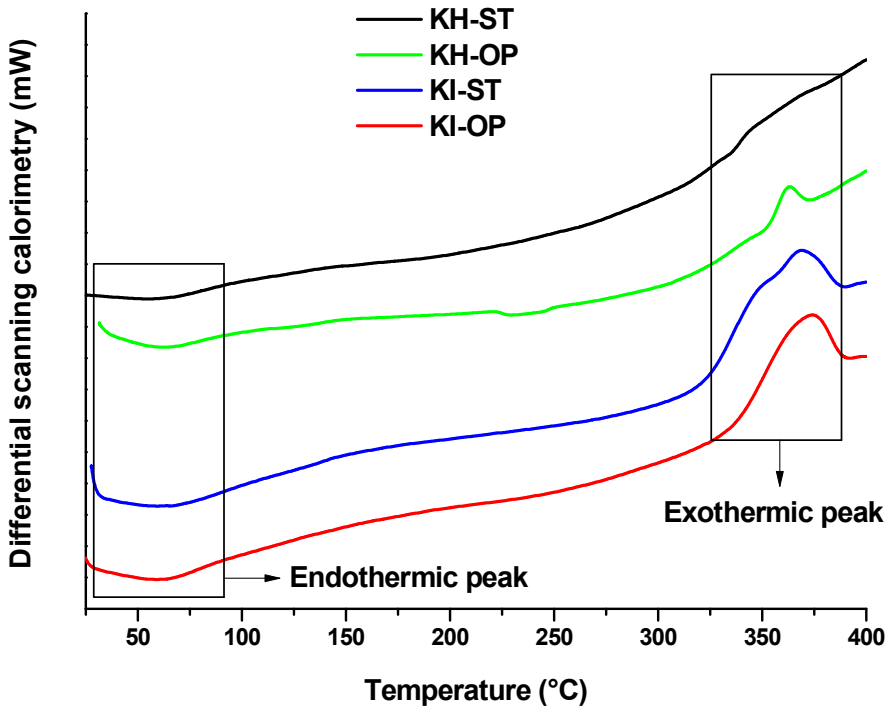
Figure 6. XRD pattern of the bacterial cellulose produced in standard and optimized medium by AAB strains *K. hansenii* ATCC 23769 **(a)** and *K. intermedius* V-05 **(b).**



4.2.3.7. DSC analysis

According to Figure 7, two main peaks are observed in DSC curves obtained from the samples produced by the strains. The endothermic peak observed around 50-75 ºC was attributed to evaporation of residual molecules of water from the polymeric matrix. The exothermic peak is observed around 360-370 ºC and was attributed to the thermal decomposition (pyrolysis) of the cellulose due to fragmentation of carbonyl and carboxylic bonds (BARUD et al., 2007). Similar results were observed by other authors in DSC analysis of pure, acetylated and phosphorylated BC membranes (BARUD et al., 2007; BARUD et al., 2008).

Figure 7. DSC curves of bacterial cellulose membranes produced by AAB strains *K. hansenii* ATCC 23769 **(KH)** and *K. intermedius* V-05 **(KI)** in standard **(ST)** and optimized **(OP)** media.

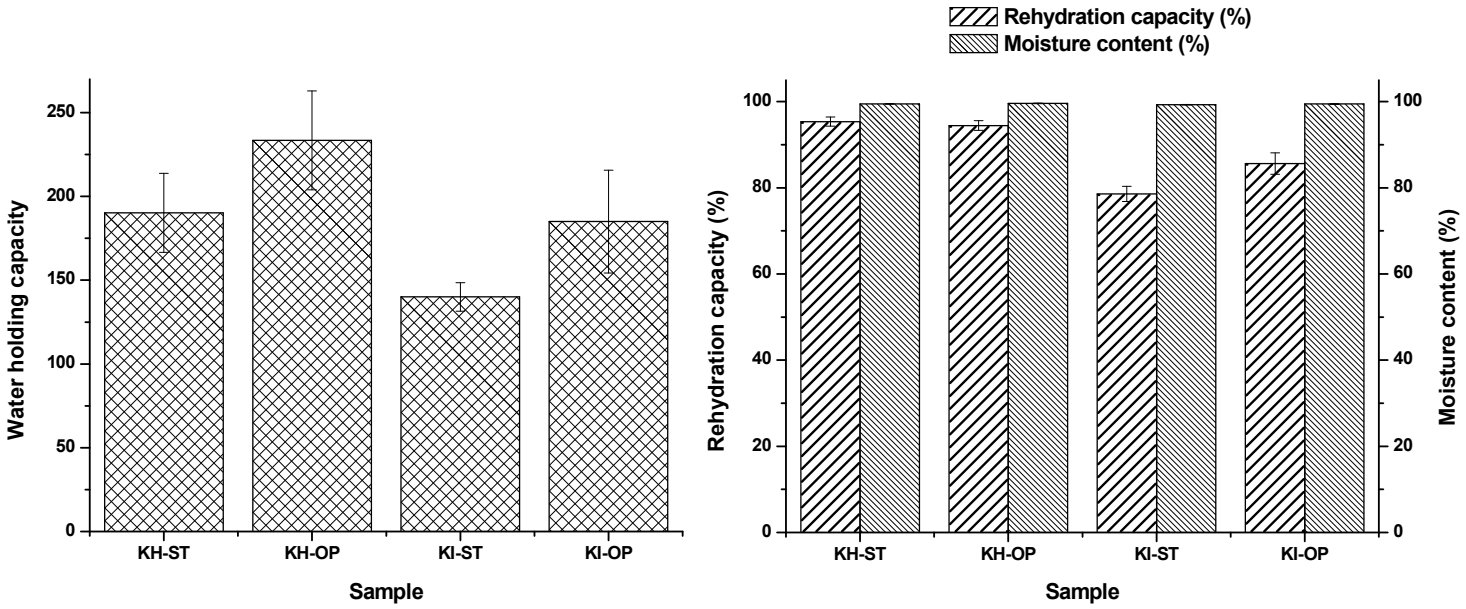


4.2.3.8. Hydrophilic properties

Figure 8 presents the characterization of the BC membranes regarding water holding capacity (WHC), rehydration capacity (RC) and moisture content. All the samples had water content superior to 99.0% (w/w).

Membranes produced in optimized medium exhibited higher WHC than those produced in HS medium by the same strain. Optimized medium also provided membranes with higher RC than those produced from HS medium by the strain *K. intermedius* V-05. However, the same improvement was not observed by *K. hansenii* 23769. The WHC represents the water mass retained per unit of cellulose dry weight. Several factors interfere in the hydrophilic properties of the BC membranes such as porosity and surface area (UL-ISLAM; KHAN; PARK, 2012); roughness or shrinkage after drying step (AMIN; ABADI; KATAS, 2014; ZENG; LAROMAINE; ROIG, 2014); and the increase of polar groups in the cellulose network (BHUMKAR; POKHARKAR, 2006). WHC is related to never-dried BC while RC represents the degree to which removed water was recovered by the samples. Drying process improves the storage and sell-life of BC, but poor rehydration capacity reduces the utility of dried BC (HUANG et al., 2010; LIN et al., 2009). In this work, improvement of WHC and RC for KI-OP samples may be correlated to the increase of empty spaces among BC fibrils, for the reason that these membranes had lower dry weight and crystallinity index providing more space for water accommodation inside the porous matrix. The observed improvement of WHC for KH-OP samples may be correlated to the increase of fibrils and hydroxyl groups within the reticulated matrix that increases the capability to make hydrogen bonds with the water. According to Eslahi et al. (2020), the high water holding capacity makes BC an excellent wound healing material by maintaining a moist environment and helping to eliminate exudates.

Figure 8. Hydrophilic properties of the cellulose membranes produced by AAB strains *K. hansenii* ATCC 23769 **(KH)** and *K. intermedius* V-05 **(KI)** in standard **(ST)** and optimized **(OP)** media.



4.2.4. Conclusion

Through this work, it can be concluded that different strains belonging to the same genus may have different nutritional needs in addition to different biosynthesis capacities of BC, depending on the composition of the used culture medium.

The Plackett and Burman factorial design followed by response surface methodology were effective in selecting and determining the best concentration of essential minerals and vitamins that affect BC production by AAB strains belonging to the genus *Komagataeibacter*. By using statistical methodologies, minerals iron, zinc and manganese, and vitamins pantothenic acid and nicotinic acid were the main micronutrients required by the strains. The response surface plots and the predictive model provided important information regarding the optimum level of each variable, the interactions between them and their effects on BC production yield.

For a low-producing strain, the optimized synthetic medium increased BC production in comparison with the non-optimized medium. Besides that, it was improved crystallinity index and thermal properties of the samples. Conversely, for a high-producing strain, BC production in optimized synthetic medium did not increased production yield but improved thermal and hydrophilic properties.

Acknowledgements

We would like to thank “Laboratório de Espectroscopia (ESPEC)” and “Laboratório de Difração de Raios X (LabDRX)” of the State University of Londrina for providing equipments and assistance in the analyses of chemical characterization. We also thank “Embrapa Tropical Agroindustry” for providing financial support and “Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)” for providing scholarship to Rodrigo Gomes (Process number: 142380/2017-2).

4.2.5. References

AJE, O. F.; ADIE, A. J. Response surface methodology and Taguchi method based applications–A Review. **Global Journal of Engineering and Technology Advances,** v. 5, n. 1, p. 053-059, 2020. https://doi.org/10.30574/gjeta.2020.5.1.0062

ALMEIDA, D. M.; PRESTES, R. A.; FONSECA, A. F. D.; WOICIECHOWSKI, A. L.; WOSIACKI, G. Minerals consumption by *Acetobacter xylinum* on cultivation medium on coconut water. **Brazilian Journal of Microbiology,** v. 44, n. 1, p. 197-206, abr. 2013. https://doi.org/10.1590/S1517-83822013005000012

ALTERTHUMM, F. Elementos de Microbiologia. In.: BORZANI, W.; SCHMIDELL, W.; LIMA, U. A.; AQUARONE, E. **Biotecnolgia Industrial:** Fundamentos. 2. ed. São Paulo: Blucher, 2020. v. 1, p. 1-32.

AMIN, M. C. I. M.; ABADI, A. G.; KATAS, H. Purification, characterization and comparative studies of spray-dried bacterial cellulose microparticles. **Carbohydrate Polymers,** v. 99, p. 180-189, jan. 2014. https://doi.org/10.1016/j.carbpol.2013.08.041

ARCHIBALD, F. Manganese: its acquisition by and function in the lactic acid bacteria. **Critical Reviews in Microbiology,** v. 13, n. 1, p. 63-109, 1986. https://doi.org/10.3109/10408418609108735.

AVERIANOVA, L. A.; BALABANOVA, L. A.; SON, O. M.; PODVOLOTSKAYA, A. B.; TEKUTYEVA, L. A. Production of vitamin B2 (riboflavin) by microorganisms: an overview. **Frontiers in Bioengineering and Biotechnology,** v. 8, nov. 2020. https://doi.org/10.3389/fbioe.2020.570828

BARUD, H.; RIBEIRO, C.; CRESPI, M.; MARTINES, M.; DEXPERT-GHYS, J.; MARQUES, R.; MESSADDEQ, Y.; RIBEIRO, S. Thermal characterization of bacterial cellulose–phosphate composite membranes. **Journal of Thermal Analysis and Calorimetry,** v. 87, n. 3, p. 815-818, mar. 2007. https://doi.org/10.1007/s10973-006-8170-5

BARUD, H. S.; ARAÚJO JÚNIOR, A. M.; SANTOS, D. B.; ASSUNÇÃO, R. M. N.; MEIRELES, C. S.; CERQUEIRA, D. A.; RODRIGUES FILHO, G.; RIBEIRO, C. A.; MESSADDEQ Y.; RIBEIRO, S. J. L. Thermal behavior of cellulose acetate produced from homogeneous acetylation of bacterial cellulose. **Thermochimica Acta,** v. 471, n. 1-2, p. 61-69, maio 2008. https://doi.org/10.1016/j.tca.2008.02.009

BERINGHS, A. O.; DALMINA, M.; CRECZYNSKI-PASA, T. B.; SONAGLIO, D. Response Surface Methodology IV-Optimal design applied to the performance improvement of an RP-HPLC-UV method for the quantification of phenolic acids in Cecropia glaziovii products. **Revista Brasileira de Farmacognosia,** v. 25, n. 5, p. 513-521, set./out. 2015. https://doi.org/10.1016/j.bjp.2015.05.007

BHUMKAR, D. R.; POKHARKAR, V. B. Studies on effect of pH on cross-linking of chitosan with sodium tripolyphosphate: A technical note. **AAPS PharmSciTech,** v. 7, n. 2, p. E138-E143, jun. 2006. https://doi.org/10.1208/pt070250

BLANCO PARTE, F. G.; SANTOSO, S. P.; CHOU, C. C.; VERMA, V.; WANG, H. T.; ISMADJI, S.; CHENG, K. C. Current progress on the production, modification, and applications of bacterial cellulose. **Critical Reviews in Biotechnology,** v. 40, n. 3, p. 397-414, 2020. https://doi.org/10.1080/07388551.2020.1713721

BIE, X.; LU, Z.; LU, F.; ZENG, X. Screening the main factors affecting extraction of the antimicrobial substance from *Bacillus* sp. fmbJ using the Plackett–Burman method. **World Journal of Microbiology and Biotechnology,** v. 21, n. 6-7, p. 925-928, out. 2005. https://doi.org/10.1007/s11274-004-6722-z

BOUDRAHEM, F.; ZIANI, S.; AISSANI-BENISSAD, F. Application of response surface methodology based on central composite design for optimization of yellow bezacryl sorption on shoe soles waste. **Environmental Progress & Sustainable Energy,** v. 38, n. s1, p. S412-S421, mar. 2019. https://doi.org/10.1002/ep.13073

BRAIDY, N.; BERG, J.; CLEMENT, J.; KHORSHIDI, F.; POLJAK, A.; JAYASENA, T.; GRANT, R.; SACHDEV, P. Role of nicotinamide adenine dinucleotide and related precursors as therapeutic targets for age-related degenerative diseases: rationale, biochemistry, pharmacokinetics, and outcomes. **Antioxidants & Redox Signaling,** v. 30, n. 2, p. 251-294, jan. 2019. https://doi.org/10.1089/ars.2017.7269

CHANDRASEKARAN, P. T.; BARI, N. K.; SINHA, S. Enhanced bacterial cellulose production from *Gluconobacter xylinus* using super optimal broth. **Cellulose,** v. 24, n. 10, p. 4367-4381, out. 2017. https://doi.org/10.1007/s10570-017-1419-2

CHASAPIS, C. T.; NTOUPA, P. S. A.; SPILIOPOULOU, C. A.; STEFANIDOU, M. E. Recent aspects of the effects of zinc on human health. **Archives of Toxicology,** v. 94, p. 1443-1460, maio 2020. https://doi.org/10.1007/s00204-020-02702-9

CHENG, Z.; YANG, R.; LIU, X.; LIU, X.; CHEN, H. Green synthesis of bacterial cellulose via acetic acid pre-hydrolysis liquor of agricultural corn stalk used as carbon source. **Bioresource Technology,** v. 234, p. 8-14, jun. 2017. https://doi.org/10.1016/j.biortech.2017.02.131

CHINA, S. L.; ZANICHELLI, G.; DE VERO, L.; GULLO, M. Oxidative fermentations and exopolysaccharides production by acetic acid bacteria: a mini review. **Biotechnology Letters,** v. 40, n. 9, p. 1289-1302, out. 2018. https://doi.org/10.1007/s10529-018-2591-7

CHINNAWIROTPISAN, P.; THEERAGOOL, G.; LIMTONG, S.; TOYAMA, H.; ADACHI, O. O.; MATSUSHITA, K. Quinoprotein alcohol dehydrogenase is involved in catabolic acetate production, while NAD-dependent alcohol dehydrogenase in ethanol assimilation in Acetobacter pasteurianus SKU1108. **Journal of Bioscience**

and Bioengineering, v. 96, n. 6, p. 564-571, 2003. https://doi.org/10.1016/S1389-1723(04)70150-4

CISTERNAS, I. S.; SALAZAR, J. C.; GARCÍA-ANGULO, V. A. Overview on the bacterial iron-riboflavin metabolic axis. **Frontiers in Microbiology**, v. 9, p. 1478, jul. 2018. https://doi.org/10.3389/fmicb.2018.01478

DU, L.; LIU, S.; HAO, G.; ZHANG, L.; ZHOU, M.; BAO, Y.; DING, B.; SUN, Q.; ZHANG, G. Preparation and release profiles *in vitro/vivo* of galantamine pamoate loaded Poly (Lactideco-Glycolide)(PLGA) microspheres. **Frontiers in Pharmacology,** v. 11, p. 2392, mar. 2021. https://doi.org/10.3389/fphar.2020.619327

ESLAHI, N.; MAHMOODI, A.; MAHMOUDI, N.; ZANDI, N.; SIMCHI, A. Processing and properties of nanofibrous bacterial cellulose-containing polymer composites: A review of recent advances for biomedical applications. **Polymer Reviews,** v. 60, n. 1, p. 144-170, 2020. https://doi.org/10.1080/15583724.2019.1663210

FENG, X.; ULLAH, N.; WANG, X.; SUN, X.; LI, C.; BAI, Y.; CHEN, L.; LI, Z. Characterization of bacterial cellulose by *Gluconacetobacter hansenii* CGMCC 3917. **Journal of Food Science,** v. 80, n. 10, p. E2217-E2227, out. 2015. https://doi.org/10.1111/1750-3841.13010

FRAWLEY, E. R.; FANG, F. C. The ins and outs of bacterial Iron metabolism. **Molecular Microbiology,** v. 93, n. 4, p. 609-616, ago. 2014. https://doi.org/10.1111/mmi.12709

GIBSON, L. J. The hierarchical structure and mechanics of plant materials. **Journal of the Royal Society Interface,** v. 12, n. 9, p. 2749-2766, set. 2012. https://doi.org/10.1098/rsif.2012.0341

GOMES, R. J.; BORGES, M. F.; ROSA, M. F.; CASTRO-GÓMEZ, R. J. H.; SPINOSA, W. A. Acetic acid bacteria in the food industry: Systematics, characteristics and applications. **Food Technology and Biotechnology,** v. 56, n. 2, p. 139-151, abr./jun. 2018. https://doi.org/10.17113/ftb.56.02.18.5593

GOMES, R. J.; FARIA-TISCHER, P. C. S.; TISCHER, C. A., CONSTANTINO, L. V.; ROSA, M. F.; CHIDEROLI, R. T.; PEREIRA, U. P.; SPINOSA, W. A. *Komagataeibacter intermedius* V-05: an acetic acid bacterium isolated from vinegar industry, with high capacity for bacterial cellulose production in soybean molasses medium. **Food Technology and Biotechnology,** v. 59, n. 4, out./dez. 2021. https://doi.org/10.17113/ftb.59.04.21.7148

GÜNDOĞDU, T. K.; DENIZ, I.; ÇALIŞKAN, G.; ŞAHIN, E. S.; AZBAR, N. Experimental design methods for bioengineering applications. **Critical Reviews in Biotechnology,** v. 36, n. 2, p. 368-388, 2016. https://doi.org/10.3109/07388551.2014.973014

HAMMAMI, A.; BAYOUDH, A.; HADRICH, B.; ABDELHEDI, O.; JRIDI, M.; NASRI, M. Response-surface methodology for the production and the purification of a new H2O2-tolerant alkaline protease from *Bacillus invictae* AH1 strain. **Biotechnology Progress,** v. 36, n. 3, p. e2965, mai./jun. 2020. https://doi.org/10.1002/btpr.2965

HESTRIN, S.; SCHRAMM, M. Synthesis of cellulose by *Acetobacter xylinum*. 2. Preparation of freeze-dried cells capable of polymerizing glucose to cellulose. **Biochemical Journal,** v. 58, n. 2, p. 345-342, out. 1954. https://doi.org/10.1042/bj0580345

HEO, M.-S.; SON, H.-J. Development of an optimized, simple chemically defined medium for bacterial cellulose production by *Acetobacter* sp. A9 in shaking cultures. **Biotechnology and applied biochemistry,** v. 36, n. 1, p. 41-45, ago. 2002. https://doi.org/10.1042/BA20020018

HOSNY, K. M.; AHMED, O. A.; FAHMY, U. A.; ALKHALIDI, H. M. Nanovesicular systems loaded with a recently approved second generation type-5 phospodiesterase inhibitor (avanafil): I. Plackett-Burman screening and characterization. **Journal of Drug Delivery Science and Technology,** v. 43, p. 154-159, fev. 2018. https://doi.org/10.1016/j.jddst.2017.10.009

HUANG, H.-C.; CHEN, L.-C.; LIN, S.-B.; HSU, C.-P.; CHEN, H.-H. In situ modification of bacterial cellulose network structure by adding interfering substances during fermentation. **Bioresource Technology,** v. 101, n. 15, p. 6084-6091, ago. 2010. https://doi.org/10.1016/j.biortech.2010.03.031

ISHIKAWA, A.; MATSUOKA, M.; TSUCHIDA, T.; YOSHINAGA, F. Increase in cellulose production by sulfaguanidine-resistant mutants derived from *Acetobacter xylinum* subsp. *sucrofermentans*. **Journal Bioscience, Biotechnology, and Biochemistry,** v. 59, n. 12, p. 2259-2262, 1995. https://doi.org/10.1271/bbb.59.2259

ISMY, J.; SUGANDI, S.; RACHMADI, D.; HARDJOWIJOTO, S.; MUSTAFA, A. The Effect of Exogenous Superoxide Dismutase (SOD) on Caspase-3 Activation and Apoptosis Induction in Pc-3 Prostate Cancer Cells. **Research and Reports in Urology,** v. 12, p. 503-508, out. 2020. https://doi.org/10.2147/RRU.S271203

JAGANNATH, A.; KALAISELVAN, A.; MANJUNATHA, S. S.; RAJU, P. S.; BAWA, A. S. The effect of pH, sucrose and ammonium sulphate concentrations on the production of bacterial cellulose (Nata-de-coco) by Acetobacter xylinum. **World**

Journal of Microbiology and Biotechnology, v. 24, n. 11, p. 2593-2599, 2008. https://doi.org/10.1007/s11274-008-9781-8

JO, J. H.; LEE, D. S.; PARK, D.; CHOE, W.-S.; PARK, J. M. Optimization of key process variables for enhanced hydrogen production by *Enterobacter aerogenes* using statistical methods. **Bioresource Technology,** v. 99, n. 6, p. 2061-2066, abr. 2008. https://doi.org/10.1016/j.biortech.2007.04.027

KAČURÁKOVÁ, M.; SMITH, A. C.; GIDLEY, M. J.; WILSON, R. H. Molecular interactions in bacterial cellulose composites studied by 1D FT-IR and dynamic 2D FT-IR spectroscopy. **Carbohydrate Research,** v. 337, n. 12, p. 1145-1153, 2002.

KANDARI, D.; JOSHI, H.; BHATNAGAR, R. Zur: Zinc-sensing transcriptional regulator in a diverse set of bacterial species. **Pathogens,** v. 10, n. 3, p. 344, 2021. https://doi.org/10.3390/pathogens10030344

KANDARI, D.; GOPALANI, M.; GUPTA, M.; JOSHI, H.; BHATNAGAR, S.; BHATNAGAR, R. Identification, functional characterization, and regulon prediction of the zinc uptake regulator (Zur) of Bacillus anthracis–an insight into the zinc homeostasis of the pathogen. **Frontiers in Microbiology,** v. 9, p. 3314, jan. 2019. https://doi.org/10.3389/fmicb.2018.03314

KATSYUBA, E.; AUWERX, J. Modulating NAD^+ metabolism, from bench to bedside. **The EMBO Journal,** v. 36, n. 18, p. 2670-2683, set. 2017. https://doi.org/10.15252/embj.201797135

KESHK, S. M. A. S. Vitamin C enhances bacterial cellulose production in *Gluconacetobacter xylinus*. **Carbohydrate polymers,** v. 99, p. 98-100, jan. 2014. https://doi.org/10.1016/j.carbpol.2013.08.060

KIRKLAND, J. B.; MEYER-FICCA, M. L. Niacin. In: ESKIN, M. N. A. **New Research and Developments of Water-Soluble Vitamins.** Cambrige: Academic Press, 2018. v. 83, p. 83–149. https://doi.org/10.1016/bs.afnr.2017.11.003

KONDO, T. The assigment of IR absorption bands due to free hidroxyl groups in cellulose. **Cellulose,** v. 4, p. 281-292, dez. 1997.

KOUR, R.; JAIN, D.; BHOJIYA, A. A.; SUKHWAL, A.; SANADHYA, S.; SAHEEWALA, H.; JAT, G.; SINGH, A.; MOHANTY, S. R. Zinc biosorption, biochemical and molecular characterization of plant growth-promoting zinc-tolerant bacteria. **3 Biotech,** v. 9, n. 11, p. 1-17, out. 2019. https://doi.org/10.1007/s13205-019-1959-2

LANSKA, D. J. The discovery of niacin, biotin, and pantothenic acid. **Annals of Nutrition and Metabolism,** v. 61, n. 3, p. 246-253, nov. 2012. https://doi.org/10.1159/000343115

LEONARDI, R.; JACKOWSKI, S. Biosynthesis of pantothenic acid and coenzyme A. **EcoSal Plus,** v. 2, n. 2, abr. 2007. https://doi.org/10.1128/ecosalplus.3.6.3.4

LEONARDI, R.; ROACH, P. L. Thiamine biosynthesis in *Escherichia coli*: in vitro reconstitution of the thiazole synthase activity. **Journal of Biological Chemistry,** v. 279, n. 17, p. 17054-17062, abr. 2004. https://doi.org/10.1074/jbc.M312714200

LI, C.; ZHOU, H.-M. The role of manganese superoxide dismutase in inflammation defense. **Enzyme Research,** v. 2011, p. 387176, out. 2011. https://doi.org/10.4061/2011/387176

LI, Y.; TIAN, C.; TIAN, H.; ZHANG, J.; HE, X.; PING, W.; LEI, H. Improvement of bacterial cellulose production by manipulating the metabolic pathways in which ethanol and sodium citrate involved. **Applied Microbiology and Biotechnology,** v. 96, n. 6, p. 1479-1487, dez. 2012. https://doi.org/10.1007/s00253-012-4242-6

LI, Z.; WANG, L.; HUA, J.; JIA, S.; ZHANG, J.; LIU, H. Production of nano bacterial cellulose from waste water of candied jujube-processing industry using *Acetobacter xylinum*. **Carbohydrate Polymers,** v. 120, p. 115-119, abr. 2015. https://doi.org/10.1016/j.carbpol.2014.11.061

LI, X.; LI, J.; GONG, J.; KUANG, Y.; MO, L.; SONG, T. Cellulose nanocrystals (CNCs) with different crystalline allomorph for oil in water Pickering emulsions. **Carbohydrate Polymers,** v. 183, p. 303-310, mar. 2018. https://doi.org/10.1016/j.carbpol.2017.12.085

LIN, S. B.; HSU, C. P.; CHEN, L. C.; CHEN, H. H. Adding enzymatically modified gelatin to enhance the rehydration abilities and mechanical properties of bacterial cellulose. **Food Hydrocolloids,** v. 23, n. 8, p. 2195-2203, dez. 2009. https://doi.org/10.1016/j.foodhyd.2009.05.011

LIN, S. P.; LIU, C. T.; HSU, K. D.; HUNG, Y. T.; SHIH, T. Y.; CHENG, K. C. Production of bacterial cellulose with various additives in a PCS rotating disk bioreactor and its material property analysis. **Cellulose,** v. 23, n. 1, p. 367-377, fev. 2016. https://doi.org/10.1007/s10570-015-0855-0

LOTFIMAN, S.; BIAK, D. R. A.; TI, T. B.; KAMARUDIN, S.; NIKBIN, S. Influence of date syrup as a carbon source on bacterial cellulose production by *Acetobacter xylinum* 0416. **Advances in Polymer Technology,** v. 37, n. 4, p. 1085-1091, jun. 2018. https://doi.org/10.1002/adv.21759

MADIGAN, M. T.; MARTINKO, J. M.; BENDER, K. S.; BUCKLEY, D. H.; STAHL, D. A. **Microbiologia de Brock.** 14 ed. Porto Alegre: Artmed, 2016.

MAKAROV, M. V.; TRAMMELL, S. A. J.; MIGAUD, M. E. The chemistry of the vitamin B3 metabolome. **Biochemical Society Transactions,** v. 47, n. 1, p. 131-147, fev. 2019. https://doi.org/10.1042/BST20180420

MAMLOUK, D.; GULLO, M. Acetic acid bacteria: physiology and carbon sources oxidation. **Indian Journal of Microbiology,** v. 53, n. 4, p. 377-384, out./dez. 2013. https://doi.org/10.1007/s12088-013-0414-z

MANDENIUS, C.-F.; BRUNDIN, A. Bioprocess optimization using design-of-experiments methodology. **Biotechnology Progress,** v. 24, n. 6, p. 1191-1203, nov./dez. 2008. https://doi.org/10.1002/btpr.67

MATSUOKA, M.; TSUCHIDA, T.; MATSUSHITA, K.; ADACHI, O.; YOSHINAGA, F. A synthetic medium for bacterial cellulose production by *Acetobacter xylinum* subsp. *sucrofermentans*. **Bioscience, Biotechnology, and Biochemistry,** v. 60, n. 4, p. 575-579, jan. 1996. https://doi.org/10.1271/bbb.60.575

MARTÍNEZ-SANZ, M.; LOPEZ-RUBIO, A.; LAGARON, J. M. Optimization of the nanofabrication by acid hydrolysis of bacterial cellulose nanowhiskers. **Carbohydrate Polymers,** v. 85, n. 1, p. 228-236, abr. 2011. https://doi.org/10.1016/j.carbpol.2011.02.021

MIKKELSEN, D.; FLANAGAN, B. M.; DYKES, G. A.; GIDLEY, M. J. Influence of different carbon sources on bacterial cellulose production by *Gluconacetobacter xylinus* strain ATCC 53524. **Jounal of Applied Microbiology,** v. 107, n. 2, p. 576-583, ago. 2009. https://doi.org/10.1111/j.1365-2672.2009.04226.x

MOHITE, B. V.; KAMALJA, K. K.; PATIL, S. V. Statistical optimization of culture conditions for enhanced bacterial cellulose production by *Gluconoacetobacter hansenii* NCIM 2529. **Cellulose,** v. 19, n. 5, p. 1655-1666, out. 2012. https://doi.org/10.1007/s10570-012-9760-y

MOLINA-RAMÍREZ, C.; CASTRO, M.; OSORIO, M.; TORRES-TABORDA, M.; GÓMEZ, B.; ZULUAGA, R.; GÓMEZ, C.; GAÑÁN, P.; ROJAS, O. J.; CASTRO, C. Effect of different carbon sources on bacterial nanocellulose production and structure using the low ph resistant strain *Komagataeibacter medellinensis*. **Materials,** v.10, n. 639, p. 1-13, jun. 2017. https://doi.org/10.3390/ma10060639

MOLINA-RAMÍREZ, C.; CAÑAS-GUTIÉRREZ, A.; CASTRO, C.; ZULUAGA, R.; GAÑÁN, P. Effect of production process scale-up on the characteristics and properties of bacterial nanocellulose obtained from overripe Banana culture

medium. **Carbohydrate Polymers,** v. 240, p. 116341, jul. 2020. https://doi.org/10.1016/j.carbpol.2020.116341

MONDAL, S.; BOSE, B. Impact of micronutrient seed priming on germination, growth, development, nutritional status and yield aspects of plants. **Journal of Plant Nutrition,** v. 42, n. 19, p. 2577-2599, 2019. https://doi.org/10.1080/01904167.2019.1655032

NARITOMI, T.; KOUDA, T.; YANO, H.; YOSHINAGA, F. Effect of ethanol on bacterial cellulose production from fructose in continuous culture. **Journal of Fermentation and Bioengineering,** v. 85, n. 6, p. 598-603, 1998. https://doi.org/10.1016/S0922-338X(98)80012-3

NOMAN, A. E.; AL-BARHA, N. S.; SHARAF, A. A. M.; AL-MAQTARI, Q. A.; MOHEDEIN, A.; MOHAMMED, H. H. H.; CHEN, F. A novel strain of acetic acid bacteria *Gluconobacter oxydans* FBFS97 involved in riboflavin production. **Scientific Reports,** v. 10, n. 1, p. 1-17, 2020. https://doi.org/10.1038/s41598-020-70404-4

NÚÑEZ, D.; CÁCERES, R.; IDE, W.; VARAPRASAD, K.; OYARZÚN, P. An ecofriendly nanocomposite of bacterial cellulose and hydroxyapatite efficiently removes lead from water. **International Journal of Biological Macromolecules,** v. 165, p. 2711-2720, dez. 2020. https://doi.org/10.1016/j.ijbiomac.2020.10.055

NURFARAHIN, A. H.; MOHAMED, M. S.; PHANG, L. Y. Culture medium development for microbial-derived surfactants production—an overview. **Molecules,** v. 23, n. 5, p. 1049, 2018. https://doi.org/10.3390/molecules23051049

PLACKETT, R. L.; BURMAN, J. P. The design of optimum multifactorial experiments. **Biometrika,** v. 33, n. 4, p. 305-325, jun. 1946. https://doi.org/10.2307/2332195

POGORELOVA, N.; ROGACHEV, E.; DIGEL, I.; CHERNIGOVA, S.; NARDIN, D. Bacterial cellulose nanocomposites: Morphology and mechanical properties. **Materials,** v. 13, n. 12, p. 2849, 2020. https://doi.org/10.3390/ma13122849

PRAJAPATI, V. S.; TRIVEDI, U. B.; PATEL, K. C. A statistical approach for the production of thermostable and alklophilic alpha-amylase from *Bacillus amyloliquefaciens* KCP2 under solid-state fermentation. **3 Biotech,** v. 5, n. 2, p. 211-220, abr. 2015. https://doi.org/10.1007/s13205-014-0213-1

QIU, X.; ZHANG, Y.; HONG, H. Classification of acetic acid bacteria and their acid resistant mechanism. **AMB Express,** v. 11, n. 1, p. 1-15, fev. 2021. https://doi.org/10.1186/s13568-021-01189-6

RODRIGUES, M. I.; IEMMA, A. F. **Planejamento de experimentos e otimização de processos.** 3.ed. Campinas: Cárita, 2014.

ROUT, G. R.; SAHOO, S. Role of iron in plant growth and metabolism. **Reviews in Agricultural Science,** v. 3, p. 1-24, 2015. https://doi.org/10.7831/ras.3.1

RUKA, D. R.; SIMON, G. P.; DEAN, K. M. Altering the growth conditions of Gluconacetobacter xylinus to maximize the yield of bacterial cellulose. **Carbohydrate polymers,** v. 89, n. 2, p. 613-622, jun. 2012. https://doi.org/10.1016/j.carbpol.2012.03.059

SALARI, M.; KHIABANI, M. S.; MOKARRAM, R. R.; GHANBARZADEH, B.; KAFIL, H. S. Preparation and characterization of cellulose nanocrystals from bacterial cellulose produced in sugar beet molasses and cheese whey media. **International Journal of Biological Macromolecules,** v. 122, p. 280-288, fev. 2019. https://doi.org/10.1016/j.ijbiomac.2018.10.136

SALVI, D. T.; BARUD, H. D. S.; TREU-FILHO, O.; PAWLICKA, A.; MATTOS, R. I.; RAPHAEL, E.; RIBEIRO, S. J. Preparation, thermal characterization, and DFT study of the bacterial cellulose. **Journal of Thermal Analysis and Calorimetry,** v. 118, n. 1, p. 205-215, out. 2014. https://doi.org/10.1007/s10973-014-3969-y

SANTOS JUNIOR, V. D.; NIZOLI, E.; GALVAN, D.; GOMES, R. J.; BIZ, G.; RESSUTTE, J. B.; ROCHA, T. S.; SPINOSA, W. A. Micronutrient requirements and effects on cellular growth of acetic acid bacteria involved in vinegar production. **Food Science and Technology,** 2021. https://doi.org/10.1590/fst.05121

SANTOS, S. M., CARBAJO, J. M., VILLAR, J. C. The effect of carbon and nitrogen sources on bacterial cellulose prod uction and properties from *Gluconacetobacter sucrofermentans* CECT 7291 focused on its use in degraded paper restoration. **BioResources,** v. 8, n. 3, p. 3630-3645, 2013.

SCHMIDT, S. B.; HUSTED, S. The biochemical properties of manganese in plants. **Plants,** v. 8, n. 10, p. 381, 2019. https://doi.org/10.3390/plants8100381

SEGAL, L. G. J. M. A.; CREELY, J. J.; MARTIN JR, A. E.; CONRAD, C. M. An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer. **Textile Research Journal,** v. 29, n. 10, p. 786-794, out. 1959. https://doi.org/10.1177/004051755902901003

SHAO, Y.; LEI, Z.; YUAN, J.; YANG, Y.; GUO, Y.; ZHANG, B. Effect of zinc on growth performance, gut morphometry, and cecal microbial community in broilers challenged with *Salmonella enterica* serovar Typhimurium. **Journal of Microbiology,** v. 52, n. 12, p. 1002-1011, dez. 2014.

SHEDID, S. M.; SAADA, H. N.; ELTAHAWY, N. A.; HAMMAD, A. S. Curative role of pantothenic acid in brain damage of gamma irradiated rats. **Indian Journal of Clinical Biochemistry,** v. 33, n. 3, p. 314-321, jul./set. 2018. https://doi.org/10.1007/s12291-017-0683-0

SIEVERS, M.; SWINGS, J. Acetobacter. **Bergey's Manual of Systematics of Archaea and Bacteria,** p. 1-7, 2015. https://doi.org/10.1002/9781118960608.gbm00876

SINGH, V.; HAQUE, S.; NIWAS, R.; SRIVASTAVA, A.; PASUPULETI, M.; TRIPATHI, C. K. M. Strategies for fermentation medium optimization: an in-depth review. **Frontiers in microbiology,** v. 7, p. 2087, jan. 2017. https://doi.org/10.3389/fmicb.2016.02087

SINGH, A.; MANJU; YADAV, A.; BISHNOI, N. R. Statistical screening and optimization of process variables for xylanase production utilizing alkali-pretreated rice husk. **Annals of Microbiology,** v. 63, n. 1, p. 353-361, mar. 2013. https://doi.org/10.1007/s13213-012-0482-z

SON, H.-J.; KIM, H.-G.; KIM, K.-K.; KIM, H.-S.; KIM, Y.-G.; LEE, S.-J. Increased production of bacterial cellulose by *Acetobacter* sp. V6 in synthetic media under shaking culture conditions. **Bioresource Technology,** v. 86, n. 3, p. 215-219, fev. 2003. https://doi.org/10.1016/S0960-8524(02)00176-1

SOUZA, E. F.; FURTADO, M. R.; CARVALHO, C. W.; FREITAS-SILVA, O.; GOTTSCHALK, L. M. Production and characterization of *Gluconacetobacter xylinus* bacterial cellulose using cashew apple juice and soybean molasses. **International Journal of Biological Macromolecules,** v. 146, p. 285-289, mar. 2020. https://doi.org/10.1016/j.ijbiomac.2019.12.180

SPRY, C.; KIRK, K.; SALIBA, K. J. Coenzyme A biosynthesis: an antimicrobial drug target. **FEMS Microbiology Reviews,** v. 32, n. 1, p. 56-106, jan. 2008. https://doi.org/10.1111/j.1574-6976.2007.00093.x

SRIVASTAVA, S. Emerging therapeutic roles for NAD^+ metabolism in mitochondrial and age-related disorders. **Clinical and Translational Medicine,** v. 5, n. 1, p. 1-11, jul. 2016. https://doi.org/10.1186/s40169-016-0104-7

SUGIYAMA, J.; PERSSON, J.; CHANZY, H. Combined infrared and electron diffraction study of the polymorphism of native celluloses. **Macromolecules,** v. 24, n. 9, p. 2461-2466, abr. 1991.

SURMA-ŚLUSARSKA, B.; PRESLER, S.; DANIELEWICZ, D. Characteristics of bacterial cellulose obtained from *Acetobacter xylinum* culture for application in papermaking. **Fibres & Textiles in Eastern Europe,** v. 16, n. 4, p. 108-111, 2008.

TABAII, M. J.; EMTIAZI, G. Comparison of bacterial cellulose production among different strains and fermented media. **Applied Food Biotechnology,** v. 3, n. 1, p. 35-41, 2016. https://doi.org/10.22037/afb.v3i1.10582

TASHARROFI, N.; ADRANGI, S.; FAZELI, M.; RASTEGAR, H.; KHOSHAYAND, M. R.; FARAMARZI, M. A. Optimization of chitinase production by *Bacillus pumilus* using Plackett-Burman design and response surface methodology. **Iranian Journal of Pharmaceutical Research,** v. 10, n. 4, p. 759-768, 2011.

TEIXEIRA, S. R. Z.; REIS, E. M. D.; APATI, G. P.; MEIER, M. M.; NOGUEIRA, A. L.; GARCIA, M. C. F.; SCHNEIDER, A. L. S.; PEZZIN, A. P. T.; PORTO, L. M. Biosynthesis and Functionalization of Bacterial Cellulose Membranes with Cerium Nitrate and Silver Nanoparticles. **Materials Research,** v. 22, p. e20190054, 2019. https://doi.org/10.1590/1980-5373-MR-2019-0054

TERAN, M. M.; LEBLANC, A. D. M.; GIORI, G. S.; LEBLANC, J. G. Thiamine-producing lactic acid bacteria and their potential use in the prevention of neurodegenerative diseases. **Applied Microbiology and Biotechnology,** v. 105, n. 5, p. 2097-2107, mar. 2021. https://doi.org/10.1007/s00253-021-11148-7

TYLICKI, A.; ŁOTOWSKI, Z.; SIEMIENIUK, M.; RATKIEWICZ, A. Thiamine and selected thiamine antivitamins—biological activity and methods of synthesis. **Bioscience Reports,** v. 38, n. 1, p. BSR20171148, jan. 2018. https://doi.org/10.1042/bsr20171148

TORTORA, G. J.; FUNKE, B. R.; CASE, C. L. **Microbiologia.** 12 ed. Porto Alegre: Artmed, 2017.

UL-ISLAM, M.; KHAN, T.; PARK, J. K. Water holding and release properties of bacterial cellulose obtained by in situ and ex situ modification. **Carbohydrate Polymers,** v. 88, n. 2, p. 596-603, abr. 2012. https://doi.org/10.1016/j.carbpol.2012.01.006

VALERA, M. J.; TORIJA, M. J.; MAS, A.; MATEO, E. Cellulose production and cellulose synthase gene detection in acetic acid bacteria. **Applied Microbiology and Biotechnology,** v. 99, n. 3, p. 1349-1361, fev. 2015. https://doi.org/10.1007/s00253-014-6198-1

VANLINDEN, M. R.; SKOGE, R. H.; ZIEGLER, M. Discovery, metabolism and functions of NAD and NADP. **The Biochemist,** v. 37, n. 1, p. 9-13, fev. 2015. https://doi.org/10.1042/BIO03701009

VASCONCELOS, N. F.; FEITOSA, J. P. A.; GAMA, F. M. P.; MORAIS, J. P. S.; ANDRADE, F. K.; SOUZA FILHO, M. S. M.; ROSA, M. F. Bacterial cellulose nanocrystals produced under different hydrolysis conditions: Properties and morphological features. **Carbohydrate Polymers,** v. 155, p. 425-431, jan. 2017. https://doi.org/10.1016/j.carbpol.2016.08.090

VERMA, U.; NAIK, J. B.; PATIL, J. S.; YADAVA, S. K. Screening of process variables to enhance the solubility of famotidine with 2-HydroxyPropyl–β-Cyclodextrin & PVP K-30 by using Plackett–Burman design approach. **Materials Science and Engineering: C,** v. 77, p. 282-292, ago. 2017. https://doi.org/10.1016/j.msec.2017.03.238

WU, J.-M.; LIU, R.-H. Cost-effective production of bacterial cellulose in static cultures using distillery wastewater. **Journal of Bioscience and Bioengineering,** v. 115, n. 3, p. 284-290, mar. 2013. https://doi.org/10.1016/j.jbiosc.2012.09.014

YAMANAKA, S.; WATANABE, K.; KITAMURA, N.; IGUCHI, M.; MITSUHASHI, S.; NISHI, Y.; URYU, M. The structure and mechanical properties of sheets prepared from bacterial cellulose. **Journal of Materials Science,** v. 24, n. 9, p. 3141-3145, set. 1989. https://doi.org/10.1007/BF01139032

YAO, C.; CHOU, J.; WANG T.; ZHAO H.; ZHANG, B. Pantothenic acid, vitamin c, and biotin play important roles in the growth of *Lactobacillus helveticus*. **Frontiers in Microbiology,** v. 9, n. 1194, p. 1-9, jun. 2018. https://doi.org/10.3389/fmicb.2018.01194

YIANNIKOURIDES, A.; LATUNDE-DADA, G. O. A short review of iron metabolism and pathophysiology of iron disorders. **Medicines,** v. 6, n. 3, p. 85, 2019. https://doi.org/10.3390/medicines6030085

YING, W. NAD^+/NADH and $NADP^+$/NADPH in cellular functions and cell death: regulation and biological consequences. **Antioxidants & Redox Signaling,** v. 10, n. 2, p. 179-206, fev. 2008. https://doi.org/10.1089/ars.2007.1672

YOLMEH, M.; JAFARI, S. M. Applications of response surface methodology in the food industry processes. **Food and Bioprocess Technology,** v. 10, n. 3, p. 413-433, jan. 2017. https://doi.org/10.1007/s11947-016-1855-2

ZENG, M.; LAROMAINE, A.; ROIG, A. Bacterial cellulose films: influence of bacterial strain and drying route on film properties. **Cellulose,** v. 21, n. 6, p. 4455-4469, dez. 2014. https://doi.org/10.1007/s10570-014-0408-y

ZHANG, Q.; LI, Y.; XU, F.; ZHENG, M.; XI, X.; ZHANG, X.; HAN, C. Optimization of submerged fermentation medium for matrine production by *Aspergillus terreus*, an endophytic fungus harboring seeds of *Sophora flavescens*, using response surface methodology. **Mycobiology,** v. 45, n. 2, p. 90-96, 2017. https://doi.org/10.5941/MYCO.2017.45.2.90

ZHAO, B.; JIANG, H.; LIN, Z.; XU, S.; XIE, J.; ZHANG, A. Preparation of acrylamide/acrylic acid cellulose hydrogels for the adsorption of heavy metal ions. **Carbohydrate Polymers,** v. 224, p. 115022, nov. 2019. https://doi.org/10.1016/j.carbpol.2019.115022

CAPÍTULO III – EFFECTS OF AMINO ACIDS SUPPLEMENTATION ON BACTERIAL CELLULOSE PRODUCTION BY AN ACETIC ACID BACTERIUM *KOMAGATAEIBACTER INTERMEDIUS*

ABSTRACT

Bacterial cellulose (BC) is a biopolymer mainly produced by acetic acid bacteria (AAB). It has several applications in medical, pharmaceutical and food industry because of its greater properties compared to plant-origin cellulose, such as higher purity and crystallinity. The knowledge of nutritional needs of microorganisms is necessary for the nutrients to be supplied in the appropriate form and amount. The supplementation of culture media for cellular growth and biosynthesis of metabolites comprises the selection of carbon and nitrogen sources, metal ions and vitamins. The choice of different nutrients can result in different metabolic effects. In this work, it was aimed to verify the effects of amino acids supplementation in culture media for BC production by an AAB strain (*Komagataeibacter intermedius* V-05), and determine the optimal levels of required amino acids for the biosynthesis. For this, nineteen amino acids were tested, selected and optimized through a Plackett & Burman factorial design and central composite design to find out the optimal concentrations of each required amino acid. Membranes produced at optimal conditions were characterized in relation to chemical structure and properties X-Ray diffraction (XRD), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), infrared spectroscopy (FT-IR) and hydrophilic properties. As result, three amino acids had a significant positive effect and were required: aspartic acid (1.5 g L^{-1}), phenylalanine (1.5 g L^{-1}) and serine (3.0 g L^{-1}). Conversely, all sulfur and positively charged amino acids had a negative effect and reduced the production yield. After optimization and validation steps, a production level of 3.05 g L^{-1} was achieved. Membranes produced from optimized media by this strain present lower yield and crystallinity index but greater thermal and hydrophilic properties than those produced from standard HS medium.

Key words: fermentation; microbial nutrition; nitrogen source; experimental design; biopolymer.

4.3.1. Introduction

Cellulose is the most abundant renewable biopolymer on Earth. It is the main structural component of the primary cell wall of several plants. However, cellulose may also be produced by some algae and bacterial strains (ISLAM et al., 2017; WANG; TAVAKOLI; TANG, 2019). Bacterial cellulose (BC) is mainly synthesized by acetic acid bacteria from the genus *Komagataeibacter*. In static cultivation method, it is formed a white gelatinous membrane on the surface of the culture medium consisting of a nanofibrillated arrangement of pure cellulosic fibers that increases in thickness with increasing of cultivation time. Unlike cellulose of plant origin, BC is free of lignin, pectin and hemicellulose. Thus, its purification process is relatively simpler, cheaper and without any environmental damage (LIN et al., 2020; WANG; TAVAKOLI; TANG, 2019; SEMJONOVS et al., 2017).

Production of cellulose using microorganisms is performed through the inoculation of viable cells in a nutrient solution, which has the purpose of promoting microbial development and favoring the product formation. There are several factors that strongly influence bacterial growth and accumulation of metabolic products including physical parameters (pH and temperature) and nutritional composition of cultivation media (JAWAN et al., 2020). In these culture media, carbon and nitrogen sources play an important role since microbial biomass is mainly composed by these elements; they are a fundamental energy source for microorganisms; and still are directly linked with product formation (LUTHRA et al., 2015; SINGH et al., 2017; GRAHOVAC et al., 2014; YIM; SONG; KIM, 2017). Microorganisms can utilize both inorganic and/or organic nitrogen sources. When utilizing organic nitrogen, the use of some specific amino acids can increase the productivity while unsuitable amino acids may inhibit the synthesis of metabolites. Using organic nitrogen source may also significantly influence cell growth and polysaccharides formation as compared to inorganic nitrogen because they are present in important cellular biomolecules such as proteins and nucleic acids. Proteins formed by amino acids are vital biomolecules that includes the enzymes. These biomolecules are responsible for catalyzing many biochemical reactions in a variety of biological processes in all living cells. So, the productivity of the BC could be improved depending on the type of nitrogen source used in the culture medium (AGARWAL, 2006; SINGH et al., 2017; NURFARAHIN; MOHAMED; PHANG, 2018; YIM; SONG; KIM, 2017).

Optimization of productive conditions is a very important step in fermentative processes due their high complexity and influence of many variables. Thus, investigating these several factors could increase the knowledge about the process. The classical method usually employed uses “one-factor-at-a-time”, in which the level of one factor is changed while keeping the other factors constant. It has the advantage of being simple and easy. However, the combined effect of all involved factors and their possible interactions are not considered. Conversely, statistical methods are generally preferred due to their advantages since they could minimize errors in determining the effect of parameters in a more economical manner (DINARVAND; REZAEE; FOROUGHI, 2017; KUMAR; PAKSHIRAJAN; DASU, 2009; WANG; WAN, 2009). In process that involves a large number of variables, the first step is to identify significant factors with positive or negative effects on the response of interest. Then, critical components can be eliminated or selected for further optimization. The Plackett & Burman factorial design is a suitable method for this purpose especially when there are many factors involved, since it allows to estimate the main effects with a reduced number of experiments (FERREIRA et al., 2017; EL-SAYED et al., 2016; EJAZ; AHMED; SOHAIL, 2018; WANG; WAN, 2009).

Following selection of main factors, the next step is to identify the optimal concentrations of selected parameters through optimization by response surface methodology (RSM). RSM is a collection of tools developed for the purpose of determining optimum operating conditions mainly applied in the chemical industry (MYERS; KHURI; CARTER, 1989). RSM utilizes mathematical and statistical techniques for designing experiments, building models, identifying relevant factors, studying interactions, and searching for optimal conditions. As result, the generated experimental model gives a relationship between the predicted values of independent variables and response variables that are used for constructing contour and surface plots (MOHAMMADI et al., 2016; CHATTOPADHYAY; SRIVASTAVA; BISARIA, 2002; QIU et al., 2013; YOLMEH; JAFARI, 2017). It has been proved that experimental designs are more efficient than traditional method, especially on multi-variables selection. The statistical approaches applied in fermentative process can result in improved production yields, reduced process variability, closer confirmation of the interest response to the expected requirements, and reduced development time and overall cost (LUTHRA et al., 2015; BOUDRAHEM; ZIANI; AISSANI-BENISSAD, 2019).

Because medium composition as nitrogen sources play a significant role in microbial growth and metabolite production, in this work it was aimed to evaluate the effects of medium supplementation with several amino acids on BC production by an acetic acid bacterium from the genus *Komagataeibacter*. Amino acids with positive effect were selected for optimization by RSM and the membranes obtained under optimal conditions were characterized in relation to its structure and properties.

4.3.2. Material and Methods

4.3.2.1. Material

The experiments were carried out by using an acetic acid bacterium named *Komagataeibacter intermedius* V-05, previously isolated from vinegar industry (GOMES et al., 2021). The amino acids were purchased from: Dinâmica (Indaiatuba, SP, Brazil), Sigma-Aldrich (St. Louis, MO, USA), Synth (Diadema, SP, Brazil), and Thermo Fisher Scientific (Waltham, MA, USA). Others reagents and chemical products were of analytical grade and purchased from different companies: Himedia (Mumbai, India), Anidrol (Diadema, SP, Brazil), Inlab (Diadema, SP, Brazil), Nuclear (Diadema, SP, Brazil) and Vetec Química (Rio de Janeiro, RJ, Brazil).

4.3.2.2. Activation of microorganisms

Bacterial colonies of *K. intermedius* V-05 previously grown on mannitol–yeast extract–peptone (MYP) agar (25 g L^{-1} mannitol, 5 g L^{-1} yeast extract, 3 g L^{-1} peptone and 10 g L^{-1} agar), were cultivated in tubes containing 10 mL HS medium, composed of 20 g L^{-1} glucose, 5 g L^{-1} peptone, 5 g L^{-1} yeast extract, 2.7 g L^{-1} Na_2HPO_4 and 1.15 g L^{-1} citric acid as described by Hestrin and Schramm (1954). After microbial growth under static conditions (30 ± 0.5 °C), the cultivated broth was transferred to new flasks containing 100 mL HS medium. This new cultivated broth was used as inoculum after incubation period (30 °C for 5 days) in microbiological incubator (Tecnal TE-391) under static conditions. Then, it was carried out the hydrolysis of BC formed on the surface of each medium using 0.02% cellulase enzyme (1-2 days at 30 ± 0.5 °C) to obtain a high amount of cells per mL.

4.3.2.3. Basal medium, inoculum and culture conditions

For analyzing the effects on BC production by adding several amino acids, it was elaborated a synthetic medium containing selected macronutrients as described previously in section 4.2.2.3. After hydrolysis of inoculum, the cells were centrifuged (Eppendorf Centrifuge 5804R, Germany) for 15 min, at 5000 rpm (5 °C), for removing culture medium interferers. A cell suspension containing 10^8 colony-forming units (CFU) per mL was transferred to flaks containing basal medium (100 mL) supplemented with varying concentrations of amino acids, according to the experiments of screening and optimization.

4.3.2.4. Experimental design for selection of amino acids

For selecting amino acids that influence BC production by the strain *K. intermedius* V-05, it was employed the Plackett & Burman design. Amino acids used were: alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan and valine. It was generated an experimental design composed of 28 runs for nineteen variables (amino acids), five dummy variables and four central points. The actual and coded values of the variables are given in Table 1 and Table A6, respectively, where each factor is represented at three levels: high (+1), middle (0) and low (−1). The incorporation of dummy variable made it possible to estimate the variance of an effect (experimental error), while the addition of central point allowed to verify the repeatability of the process (RAJPUT; PATEL; TRIVEDI, 2016). The experimental design was generated and analyzed by using statistical software R® (R Core Team, Version 3.6.3). Response variable was the mass of BC produced and expressed in dry weight (g L^{-1}). The effect of each variable was equal to the difference between the average of the response variable made at the high concentration level of the factor and the average of the response variables made at the low concentration level of the same factor. The resultant effect of each variable was determined by the following equation (DU et al., 2021):

$$E(\mathbf{X}i) = 2\ \frac{(\sum \mathbf{M}i^{+} - \sum \mathbf{M}i^{-})}{N}$$

where $\boldsymbol{E(Xi)}$ is the concentration effect of the tested variable; $\mathbf{M}\boldsymbol{i}^{+}$ and $\mathbf{M}\boldsymbol{i}^{-}$ represent BC production from the runs where the measured variables $\mathbf{X}\boldsymbol{i}$ were present at high and low concentrations, respectively; and $\boldsymbol{N}$ is the total number of runs that was equal to 24. The significance level of each variable was determined via their *p*-values by using Student's t-test (SINGH et al., 2017):

$$\boldsymbol{t(Xi)} = \frac{\boldsymbol{E(Xi)}}{\mathbf{SE}}$$

where $\boldsymbol{E(Xi)}$ is the effect of variable $\mathbf{X}\boldsymbol{i}$ and **SE** is the standard error of the concentration effect that was equal to square root of the variance of an effect.

Variables with confidence levels greater than 90% were considered to influence the BC production significantly. From the analysis of results, only relevant variables with significant and positive effects were selected.

4.3.2.5. Central composite design

A 2^3 Central Composite Design (CCD) was used to obtain the optimal levels of the three selected variables (section 4.3.2.4). Independent variables were: X_1 = aspartic acid (g L^{-1}), X_2 = phenylalanine (g L^{-1}) and X_3 = serine (g L^{-1}) at five fixed levels (-α, -1, 0, +1 and +α). The full factorial design was consisted of 18 experiments performed with different combinations of the three independent variables, six axial points and four replicates at the center point (Table 2). Response variable was the mass of BC produced and expressed in dry weight (g L^{-1}). By using the statistical software R® (R Core Team, Version 3.6.3), it was generated and analyzed the experimental design as well as determined the estimated effects, regression coefficients, analysis of variance (ANOVA) and the optimal conditions of the essential amino acids. The second-order polynomial model was represented by the following quadratic equation (ZHANG et al., 2017):

$$\mathbf{Y} = \boldsymbol{\beta_0} + \sum \boldsymbol{\beta_i X_i} + \sum \boldsymbol{\beta_{ii} X_i^2} + \sum \boldsymbol{\beta_{ij} X_i X_j}$$

where: $\mathbf{Y}$ is the predicted response variable; $\boldsymbol{\beta_0}$ is the intercept coefficient; $\boldsymbol{\beta_i}$ is the coefficient of linear effect; $\boldsymbol{\beta_{ii}}$ is the coefficient of squared effect; $\boldsymbol{\beta_{ij}}$ is the coefficient of interaction; and $\mathbf{X_i}$ is the coded value of the independent variables. An experiment was run using the optimal values given by polynomial equation to confirm the predicted values and in HS medium to compare the BC production in both media.

4.3.2.6. Quantification of bacterial cellulose produced

After 10 days, the bacterial cellulose produced on the surface of each medium were collected and heated in 2% (w/v) NaOH solution for 1 h at 80 ºC and then, washed with distilled water until neutral pH (NÚÑEZ et al., 2020). The membranes obtained after the alkaline treatment were dried until constant weight, to determine the respective yields expressed in dry weight (g L^{-1}).

4.3.2.7. Characterization of bacterial cellulose produced

Bacterial cellulose produced under optimal conditions and in standard HS medium were dried in oven at 60 ºC to be characterized as follows:

4.3.2.7.1. Fourier transform infrared spectroscopy (FT-IR)

Infrared spectra were recorded using a spectrometer (Shimadzu, Prestige-21, Japan) over the 4000–400 cm^{-1} range. Dried BC samples were mixed with potassium bromide (KBr) powder and their spectra were collected in transmission mode, with a resolution of 1 cm^{-1}.

4.3.2.7.2. X-ray diffraction (XRD)

XRD patterns of the samples were obtained using a X-ray diffractometer (Malvern Panalytical X'Pert PRO MPD, Almelo, Netherlands) with Cu-Kα radiation (λ = 1.5418 Å) in the method known as θ-2θ, Bragg-Brentano geometry. The radiation source voltage and electric current were 40 kV and 40 mA, respectively. The 2θ scans were made in the range between 5 and 70º with an angular step of 0.04º.

Counting time per point was 3.0 s. The degree of crystallinity was determined for cellulose I as described by Segal et al. (1959):

$$\textbf{Crystallinity Index (\%)} = \frac{(I_{002} - Iam)}{I_{002}} \times 100$$

where I_{002} is the maximum peak intensity corresponding to the 002 lattice diffraction at angle 2θ = 22.8°; and **Iam** is the intensity of diffraction corresponding to the amorphous background at angle 2θ = 18°.

4.3.2.7.3. Differential scanning calorimetry (DSC)

DSC curves of the samples were obtained using a differential scanning calorimeter (Shimadzu DSC-60, Tokyo, Japan). The scans were started at room temperature and completed at 400 °C under a nitrogen atmosphere (50 mL min^{-1}) and heating rate of 10 °C min^{-1}. The calorimeter was calibrated with indium standard.

4.3.2.7.4. Thermogravimetric analysis (TGA)

Thermal analysis of the samples (approximately 5 mg) was performed using a thermal analyser (Shimadzu TGA-50, Tokyo, Japan). The temperatures of scans were ramped from room temperature to 600 ºC at a heating rate of 10 °C min^{-1}, under nitrogen atmosphere (50 mL min^{-1}). Derivative form of TG curves (DTG) was obtained using differential of TGA values. The TGA and DTG curves were expressed as the mass variation as a function of temperature.

4.3.2.7.5. Hydrophilic properties

For determination of the water holding capacity (WHC), rehydration capacity (RC) and moisture content, never-dried samples were shaken quickly and weighed after being removed from the storage recipient (BC_{wet}). Then, the samples were dried in oven until completely remove the water, and weighed again (BC_{dry}). WHC was calculated by the following formula (FENG et al., 2015):

$$\textbf{Water holding capacity} = \frac{(BC_{wet} - BC_{dry})}{BC_{dry}}$$

The reabsorption capacity was determined by immersion of dried membranes (BC_{dry}) in distilled water until rehydrated samples (BC_r) reached constant weight. RC was calculated by the following formula (TEIXEIRA et al., 2019):

$$\textbf{Rehydration capacity (\%)} = \frac{(BC_r - BC_{dry})}{BC_r} \textbf{ x 100}$$

The moisture content of BC was determined according to the following equation (CHANDRASEKARAN; BARI; SINHA, 2017):

$$\textbf{Moisture content (\%)} = \frac{(BC_{wet} - BC_{dry})}{BC_{wet}} \textbf{ x 100}$$

4.3.3. Results and Discussion

4.3.3.1. Effect analysis of amino acids on the bacterial cellulose production by acetic acid bacteria

Table 1 shows the Plackett & Burman experimental design containing the actual values of independent variables and the response variable corresponding to the mass of BC obtained in each one of the 28 experimental runs. The 19 amino acids evaluated in this assay produced between 0.72 to 1.40 $g.L^{-1}$ of BC dry weight. Table A7 presents the estimated effects and confidence level (*p*-value) of each variable on BC production. The coefficient of determination (R^2) was equal to 0.9468 and indicated that 94.68% of the results variability can be explained by the model. The Plackett & Burman design was useful to select the most significant variables. Among the 19 amino acids tested, aspartic acid, phenylalanine and serine had more significant positive effect and were required for the biosynthesis of BC.

Table 1. Matrix of Plackett & Burman design and observed responses of bacterial cellulose production by *K. intermedius* V-05.

Run	Independent variables (g L^{-1})																			Response variable (CB, g L^{-1})
	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}	X_{16}	X_{17}	X_{18}	X_{19}	
1	0.30	0	0	0	0	0.30	0	0.30	0	0	0.30	0.30	0	0	0.30	0.30	0	0.30	0	1.05
2	0.30	0.30	0	0	0	0	0.30	0	0.30	0	0	0.30	0.30	0	0	0.30	0.30	0	0.30	0.99
3	0.30	0.30	0.30	0	0	0	0	0.30	0	0.30	0	0	0.30	0.30	0	0	0.30	0.30	0	1.08
4	0.30	0.30	0.30	0.30	0	0	0	0	0.30	0	0.30	0	0	0.30	0.30	0	0	0.30	0.30	1.21
5	0.30	0.30	0.30	0.30	0.30	0	0	0	0	0.30	0	0.30	0	0	0.30	0.30	0	0	0.30	0.72
6	0	0.30	0.30	0.30	0.30	0.30	0	0	0	0	0.30	0	0.30	0	0	0.30	0.30	0	0	1.30
7	0.30	0	0.30	0.30	0.30	0.30	0.30	0	0	0	0	0.30	0	0.30	0	0	0.30	0.30	0	1.22
8	0	0.30	0	0.30	0.30	0.30	0.30	0.30	0	0	0	0	0.30	0	0.30	0	0	0.30	0.30	1.11
9	0.30	0	0.30	0	0.30	0.30	0.30	0.30	0.30	0	0	0	0	0.30	0	0.30	0	0	0.30	0.99
10	0.30	0.30	0	0.30	0	0.30	0.30	0.30	0.30	0.30	0	0	0	0	0.30	0	0.30	0	0	1.17
11	0	0.30	0.30	0	0.30	0	0.30	0.30	0.30	0.30	0.30	0	0	0	0	0.30	0	0.30	0	1.06
12	0	0	0.30	0.30	0	0.30	0	0.30	0.30	0.30	0.30	0.30	0	0	0	0	0.30	0	0.30	1.02
13	0.30	0	0	0.30	0.30	0	0.30	0	0.30	0.30	0.30	0.30	0.30	0	0	0	0	0.30	0	0.82
14	0.30	0.30	0	0	0.30	0.30	0	0.30	0	0.30	0.30	0.30	0.30	0.30	0	0	0	0	0.30	0.73
15	0	0.30	0.30	0	0	0.30	0.30	0	0.30	0	0.30	0.30	0.30	0.30	0.30	0	0	0	0	1.11
16	0	0	0.30	0.30	0	0	0.30	0.30	0	0.30	0	0.30	0.30	0.30	0.30	0.30	0	0	0	1.35
17	0.30	0	0	0.30	0.30	0	0	0.30	0.30	0	0.30	0	0.30	0.30	0.30	0.30	0.30	0	0	1.31
18	0	0.30	0	0	0.30	0.30	0	0	0.30	0.30	0	0.30	0	0.30	0.30	0.30	0.30	0.30	0	0.83
19	0.30	-1	0.30	0	0	0.30	0.30	0	0	0.30	0.30	0	0.30	0	0.30	0.30	0.30	0.30	0.30	1.25
20	0	0.30	0	0.30	0	0	0.30	0.30	0	0	0.30	0.30	0	0.30	0	0.30	0.30	0.30	0.30	1.16
21	0	0	0.30	0	0.30	0	0	0.30	0.30	0	0	0.30	0.30	0	0.30	0	0.30	0.30	0.30	0.78
22	0	0	0	0.30	0	0.30	0	0	0.30	0.30	0	0	0.30	0.30	0	0.30	0	0.30	0.30	1.40
23	0	0	0	0	0.30	0	0.30	0	0	0.30	0.30	0	0	0.30	0.30	0	0.30	0	0.30	1.12
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.40
25	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	1.12
26	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	1.12
27	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	1.10
28	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	1.09

Legend: X_1 – alanine; X_2 – arginine; X_3 – asparagine; X_4 – aspartic acid; X_5 – cysteine; X_6 – glycine; X_7 – glutamine; X_8 – glutamic acid; X_9 – histidine; X_{10} – leucine; X_{11} – isoleucine; X_{12} – methionine; X_{13} – proline; X_{14} – phenylalanine; X_{15} – lysine; X_{16} – serine; X_{17} – threonine; X_{18} – valine; X_{19} – tryptophan.

Aspartic acid (also known as aspartate) is a negatively charged polar amino acid constituent of most proteins and peptides. It is also used for the synthesis of asparagine, pyrimidine and purine nucleotides (KOHLMEIER, 2015; WU, 2009). Furthermore, aspartate is a precursor of β-alanine. It has been suggested that some bacteria and fungi are capable of converting β-alanine to pantothenic acid *in vivo*. The originated pantothenate is either released from the cell or used for coenzyme A (CoA) biosynthesis. CoA is an essential cofactor for the growth of microorganisms. It is involved in many metabolic reactions, including the synthesis of phospholipids, synthesis and degradation of fatty acids, and the operation of the tricarboxylic acid cycle (TCA), which is one of the stages of cellular respiration to produce energy from carbohydrate catabolism (LEONARDI; JACKOWSKI, 2007; SPRY; KIRK; SALIBA, 2008; WU, 2009; TORTORA et al., 2017). In addition to CoA, aspartate may be a key precursor for nicotinamide adenine dinucleotide (NAD^+) synthesis, which is an important cofactor for several cellular enzymes. NAD and its phosphorylated form NADP, can be originate via quinolinic acid from several routes depending on the organism and the precursors used. The major biosynthetic pathways used for quinolinate production *in vivo* are: from aspartate in most prokaryotes and from tryptophan in eukaryotic cells (SAHM et al., 2013; ZHU; PEÑA; BENNETT, 2021; VANLINDEN; SKOGE; ZIEGLER, 2015).

The aromatic amino acid phenylalanine is needed for the synthesis of proteins and is also an important precursor of the amino acid tyrosine. Catabolism of tyrosine generates fumarate that is a glucogenic intermediate utilized directly through the Krebs cycle (KOHLMEIER, 2015). Tyrosine was the only proteinogenic amino acid not used in this study due to its low solubility in water.

Serine is a neutral amino acid needed for the synthesis of proteins, selenocysteine and other amino acids such as glycine, cysteine and alanine. Serine can also be a precursor for the synthesis of several types of phospholipids, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylserine (PS) (KOHLMEIER, 2015). Phospholipids are the molecules that build cell membranes. The plasma membrane of prokaryotes consists primarily of phospholipids, which are arranged in two parallel rows, named lipid bilayer. PC is predominately found in the outer leaflet of the cell membrane, while PE and PS are predominating in the inner leaflet phospholipid. Phospholipids are essential components to the cell’s survival because they act as a barrier of membrane that

separates the cell content from the water-based environment in which it lives (PEREIRA; GIRARDI; BAKOVIC, 2012; TORTORA et al., 2017).

Significant negative effects were observed for seven amino acids: methionine ($p < 0.001$), cysteine ($p < 0.001$), arginine ($p < 0.05$), tryptophan ($p < 0.05$), alanine ($p < 0.05$), leucine ($p < 0.05$), and histidine ($p < 0.10$). If the effect found was negative and showed significance level equal or above 90% it was considered effective but the concentration required may be lower than the indicated low level (-1). So, they were removed from the optimization experiments. Remaining components, *i.e*. asparagine, glycine, glutamine, glutamic acid, isoleucine, proline, lysine, threonine and valine showed low positive or negative confidence level and were considered insignificant in this study. In a generally way, all sulfur and positively charged amino acids were rejected. This fact demonstrates that the most suitable amino acids for this strain are the non-sulfurized and not positively charged polar amino acids. Aspartic acid, phenylalanine and serine were selected to optimize their response and found their optimum levels through RSM.

Heo and Son (2002) investigated BC production by *Acetobacter* sp. A9 in a defined medium by omission of a single component. It was observed that the single omission of arginine or threonine affected BC production causing a slight decrease. In the same way, Son et al. (2003) verified that among 18 amino acids tested (except glutamine and phenylalanine), the single addition of tyrosine, valine, methionine, isoleucine, or glycine reduced the level of BC by *Acetobacter* sp. V6. However, BC production by the addition of the other amino acids slightly increased. In study about the effects of 14 amino acids (except asparagine, glutamine, histidine, cysteine, tyrosine and tryptophan), it was found that methionine stimulated the growth rate during the early stage of culture period and increased cellulose production by *Acetobacter xylinum* subsp. *sucrofermentans*. This study suggested that the amino acid methionine would be essential for obtaining high cellulose yields and stimulating the cell growth for this strain (MATSUOKA et al., 1996). These facts demonstrate that possibly due to structural similarities of amino acids, the requirements of these compounds for cellulose production by AAB may vary significantly and is strongly depend on the strain and methodology of study.

4.3.3.2. Analysis of central composite design

Table 2. Coded and actual values of the independent variables (amino acids) analyzed in the central composite design.

Run	Independent variables (g L^{-1})			Response variable (CB, g L^{-1})
	X_1	X_2	X_3	
1	-1 (0.60)	-1 (0.60)	-1 (1.2)	1.98
2	-1 (0.60)	-1 (0.60)	1 (4.8)	2.00
3	-1 (0.60)	1 (2.4)	-1 (1.2)	2.02
4	-1 (0.60)	1 (2.4)	1 (4.8)	2.30
5	1 (2.4)	-1 (0.60)	-1 (1.2)	1.97
6	1 (2.4)	-1 (0.60)	1 (4.8)	2.32
7	1 (2.4)	1 (2.4)	-1 (1.2)	2.26
8	1 (2.4)	1 (2.4)	1 (4.8)	2.25
9	-1.68 (0)	0 (1.5)	0 (3.0)	1.21
10	1.68 (3.0)	0 (1.5)	0 (3.0)	1.98
11	0 (1.5)	-1.68 (0)	0 (3.0)	1.85
12	0 (1.5)	1.68 (3.0)	0 (3.0)	2.01
13	0 (1.5)	0 (1.5)	-1.68 (0)	2.22
14	0 (1.5)	0 (1.5)	1.68 (6.0)	2.66
15	0 (1.5)	0 (1.5)	0 (3.0)	3.09
16	0 (1.5)	0 (1.5)	0 (3.0)	2.94
17	0 (1.5)	0 (1.5)	0 (3.0)	2.82
18	0 (1.5)	0 (1.5)	0 (3.0)	2.97

Legend: X_1 – aspartic acid; X_2 – phenylalanine; X_3 – serine.

Table 2 shows the central composite design involving three independent variables and the response variable expressed as mass of BC obtained in each one of the 18 experimental runs. The dry weight of BC varied from 1.21 to 3.09 g L^{-1}. Analysis of variance (Table A8) showed that all quadratic effects were negative and significant ($p < 0.10$), which indicated that the optimal region was reached. Moreover, no interaction term had significant positive or negative effect, indicating that there was no interaction among these parameters that positively or negatively influence the BC production. The coefficient of determination ($R^2 = 0.9091$) was satisfactory and indicated that 90.91% of the data variability can be explained by the predicted model. The lack-of-fit test was not significant ($p > 0.10$) indicating that the predicted model was well-adjusted to the data.

From the results achieved by central composite design, it was obtained the second-order regression model that explains the dependence of independent variables on response variable and provides the levels of BC as a function of the concentration of amino acids aspartic acid, phenylalanine and serine. So, the levels of BC produced by *K. intermedius* V-05 may be predicted by the following quadratic polynomial Equation 1, in which terms in bold are considered significant at the 90% confidence level:

$\mathbf{Y_{BC}}$ = **2.94 (± 0.10) + 0.132x_1 (± 0.057)** + 0.061x_2 (± 0.057) + 0.101x_3 (± 0.057) – **0.439x_1^2 (± 0.059) – 0.318x_2^2 (± 0.059) – 0.135x_3^2 (± 0.059)** – 0.015x_1x_2 (± 0.074) + 0.005x_1x_3 (± 0.074) – 0.013x_2x_3 (± 0.074)

where $\mathbf{Y_{BC}}$ is the response variable (BC production), x_1 is the coded value of variable X_1 (aspartic acid), x_2 is the coded value of variable X_2 (phenylalanine) and x_3 is the coded value of variable X_3 (serine). Figure A5 represents the observed values of BC production obtained in the central composite design and the predicted values determined by the Equation (1).

The three-dimensional (3D) response surface graphs and contours curves were plotted from the second-order regression model. Figure 1 shows the dependency of BC production on the variables studied at different concentration levels and the regions of maximum production. According to Figures 1(a), 1(b) and 1(c), the increase or decrease in aspartic acid, phenylalanine and serine concentration resulted in decreased BC production levels. From the quadratic polynomial Equation (1) and the response surface plots, the optimal concentration of the variables was found to be closer to the (0) level, *i.e.*, 1.50 g L^{-1} aspartic acid, 1.50 g L^{-1} phenylalanine and 3.00 g L^{-1} serine.

4.3.3.3. Validation of the experimental model

Verification of the calculated optimal conditions for BC production was done by performing the experiment at predicted optimized conditions. In this study, the maximum predicted BC production that can be obtained using the optimal concentrations of variables (1.50 g L^{-1} aspartic acid, 1.50 g L^{-1} phenylalanine and

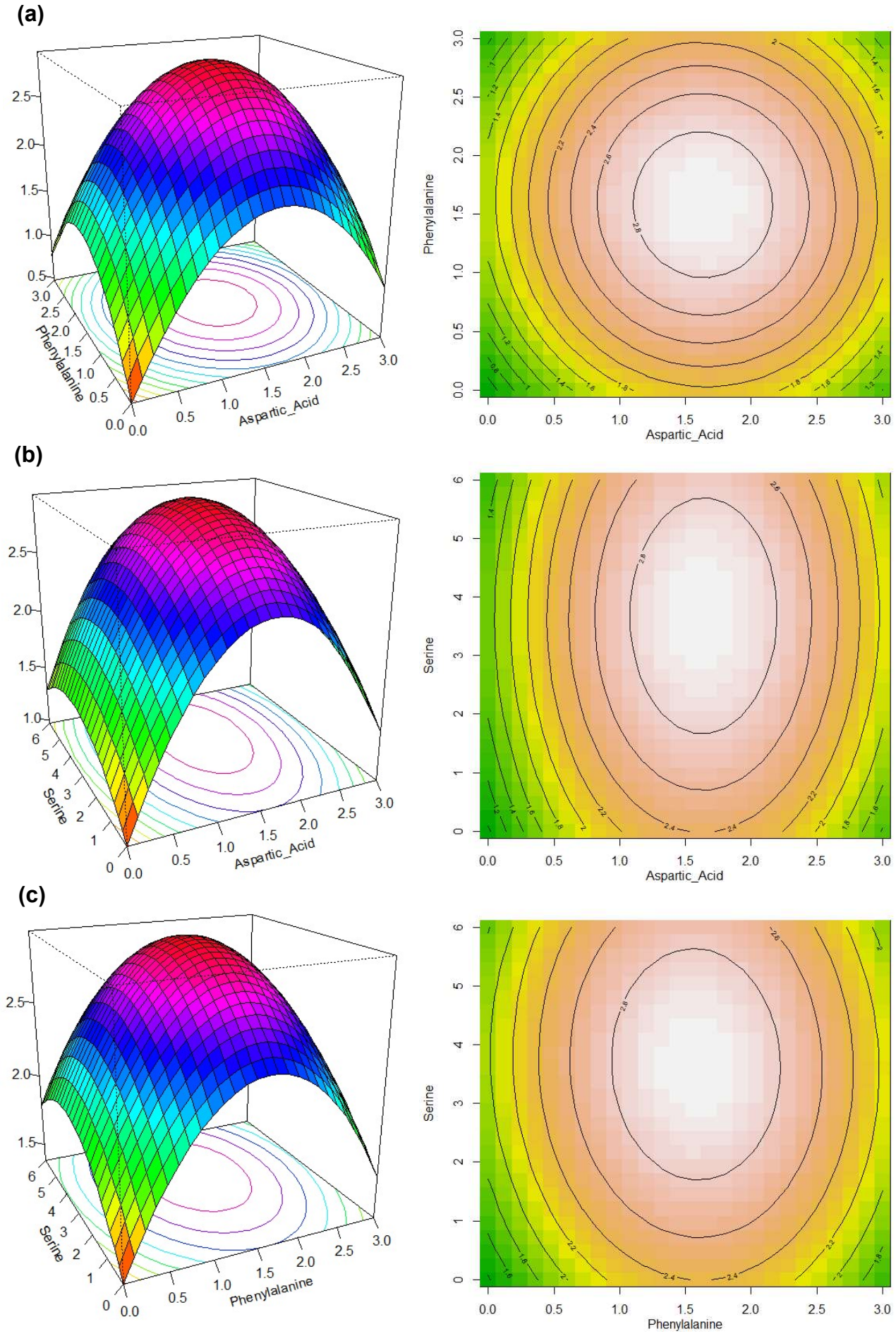


Figure 1. Contour curves for the BC production as a function of concentration (g L^{-1}) of aspartic acid and phenylalanine **(a)**, aspartic acid and serine **(b)**, and phenylalanine and serine **(c)** required by strain *K. intermedius* V-05.

3.00 g L^{-1} serine) was 2.94 g L^{-1}. The observed value for BC production were very close ($p < 0.05$) to the value estimated by the predictive model (Table 3).

Table 3. Results of validation for optimal concentration of amino acids that affect bacterial cellulose production, and results of thermal and hydrophilic properties of the membranes produced in optimized and standard media by *K. intermedius* V-05.

Medium	Maximum Production (g L^{-1})	Thermal Properties			Hydrophilic Properties*	
		T_{onset}[a] (°C)	T_{max}[b] (°C)	T_{endset}[c] (°C)	WHC[d]	RC[e] (%)
Optimized	3.02 ± 0.16	320.9	364.9	389.8	200.8[a] ± 24.0	86.3[a] ± 3.5
Standard	3.43 ± 0.19	332.5	358.5	375.0	140.0[b] ± 8.7	78.6[b] ± 1.8

* Means followed by equal letters in the same column do not differ significantly from each other based on the Tukey test ($p \leq 0.05$).
[a] = temperature of initial thermal decomposition.
[b] = temperature of maximum weight loss rate.
[c] = temperature of final thermal decomposition.
[d] = Water holding capacity.
[e] = Rehydration capacity.

4.3.3.4. FT-IR analysis

Chemical characterization of functional groups of BC produced from optimized medium was performed by FT-IR spectroscopy and compared to samples produced from standard HS medium. Figure 2 exhibits the obtained FT-IR spectra of the characterized samples which shows they present similar profiles.

According to Table 4, the samples presented the main bands that characterize the cellulose polymer, such as: strong transmission of O-H stretching vibrations at 3400-3500 cm^{-1}; alkane C-H stretching and CH_2 asymmetric stretching vibration at 2900 cm^{-1}; CH_2 symmetric stretching vibration at 2700 cm^{-1}; O-H deformation vibration at 1600 cm^{-1}; CH_2 deformation vibration at 1400 cm^{-1}; CH_3 deformation vibration at 1370 cm^{-1}; and C-O deformation vibration in the range of 1320-1030 cm^{-1} as described by Kačuráková et al. (2002), Kondo (1997) and Sugiyama et al. (1991). Bands observed at 1640 cm^{-1} (H-O-H) and 3500 cm^{-1} (O-H) were attributed to water absorption by the samples.

Absence of bands at 1735 cm^{-1} associated with functional groups present in proteins (C=O) and other bands of nitrogenous organic compounds at 1538 cm^{-1} indicated that the purification process was efficient. The results of FT-IR analysis are in agreement with studies carried out previously also indicating that the substances produced were chemically pure bacterial cellulose (POGORELOVA et al., 2020).

Figure 2. FT-IR spectra of bacterial cellulose produced by K. *intermedius* V-05 in optimized **(a)** and standard **(b)** media.

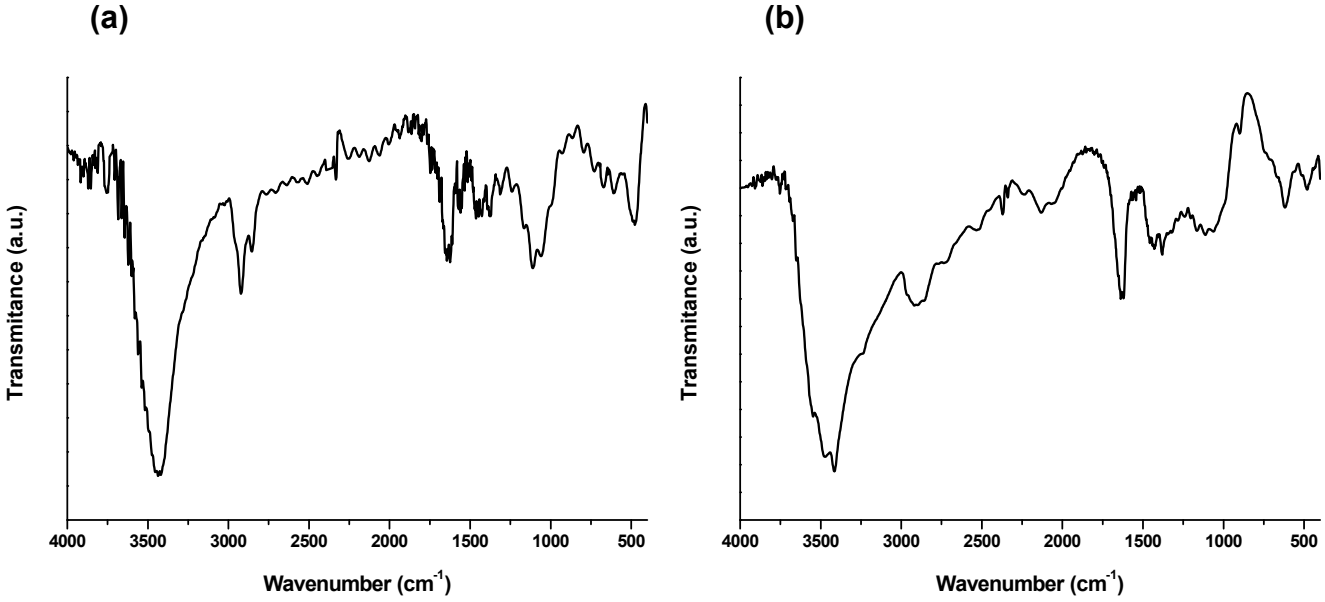


Table 4. Band assignments in the FT-IR spectra of bacterial cellulose produced by *K. intermedius* V-05 in optimized and standard media.

Band assignments	Wave number (cm^{-1})	
	Optimized	**Standard**
O-H stretching vibration	3420	3416
C-H stretching vibration	2922	2918
CH_2 stretching vibration	n.d.	2735
O-H deformation vibration	1624	1620
CH_2 deformation vibration	1427	1429
CH_3 deformation vibration	1379	1381
C-O deformation vibration	1111	1113
H-O-H bending vibration of absorbed water	1645	1638

n.d. = not detected.

4.3.3.5. XRD analysis

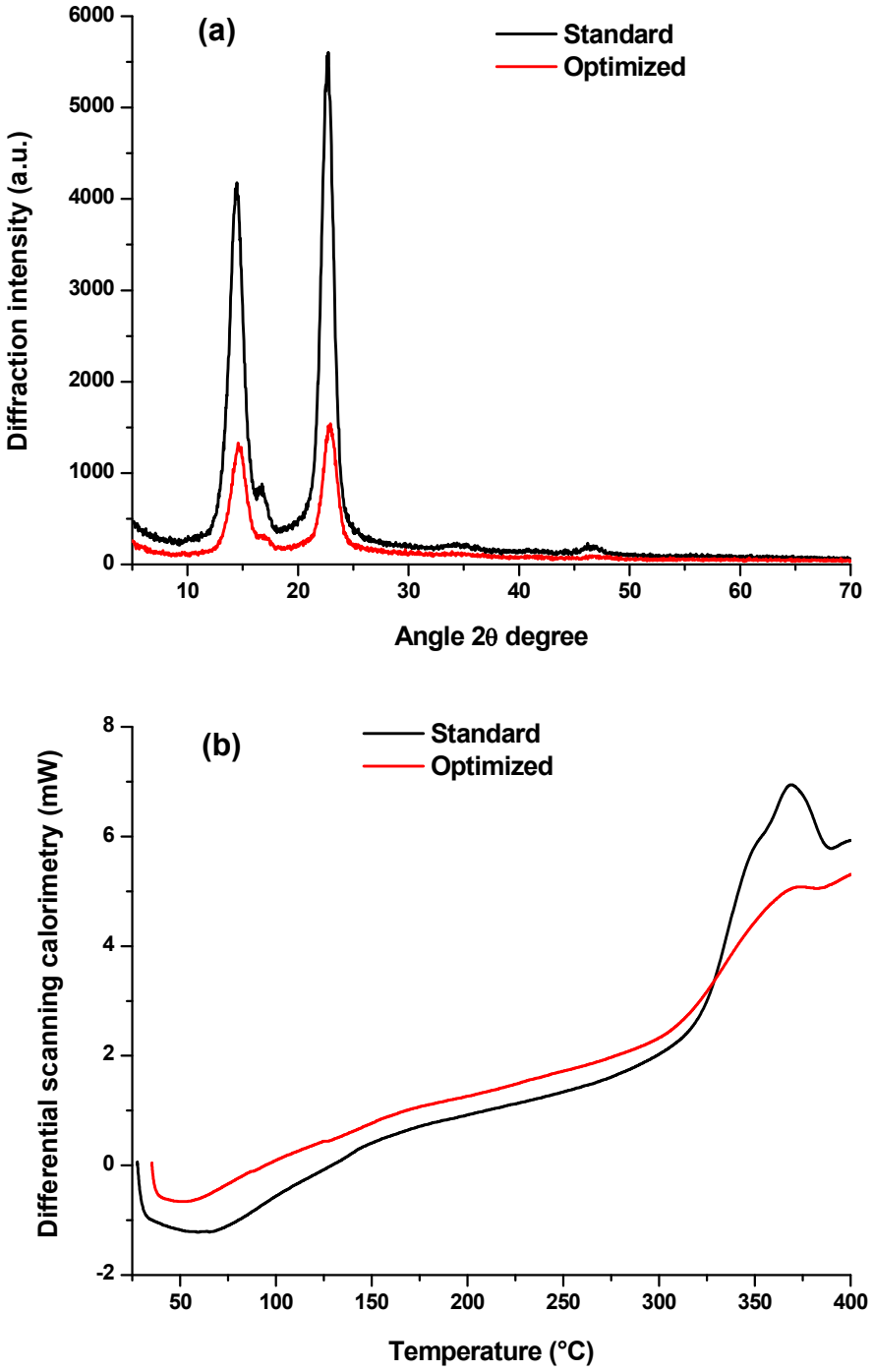
The XRD patterns of BC samples produced in both optimized and standard HS media (Figure 3(a)) demonstrated that the culture medium did not significantly influence the crystal organization of the BC membranes because they showed similar crystalline profiles. Both samples present two major peaks at 15.0º and 22.5º and a low-intensity peak at 16.8°. These diffraction peaks corresponded to the primary diffraction of the crystal planes (1-10), (200) and (110), respectively. The two main peaks observed at 14.8º and 22.5º demonstrated that the samples possess typical crystalline forms of cellulose I. According to the calculated parameters from the related peak intensity, the membranes obtained from optimized medium showed crystallinity index slightly lower (86.5%) than those from HS medium (91.0%). This fact may be related to the higher yield reached by this strain in HS compared to optimized medium (Table 3). The crystallinity index of the samples were similar to those described by Güzel and Akpinar (2020), Keshk (2014) and Sommer et al. (2021) in which crystallinity index of produced BC varied between 80.3 and 93.0%.

The high crystallinity index of the samples produced by *K. intermedius* V-05 may indicates an increase of crystalline regions and hydrophobic interactions, making these samples interesting sources for being used to stabilize water/oil (W/O) emulsions (LI et al., 2018).

4.3.3.6. DSC analysis

DSC was used to measure the thermal stability behavior of BC membranes produced in both optimized and HS standard media. Figure 3(b) shows the DSC curves containing two main peaks present in the spectra of both samples. The endothermic peak (50-65 ºC) was attributed to evaporation of residual water from the polymers, while the exothermic peak is (370 ºC) was attributed to the thermal decomposition (pyrolysis) of the cellulose due to fragmentation of carbonyl and carboxylic bonds (BARUD et al., 2007). The observed results are in accordance with other results obtained from DSC analysis of pure, acetylated and phosphorylated BC membranes reported by Barud et al. (2007) and Barud et al. (2008).

Figure 3. XRD and DSC patterns of the bacterial cellulose produced by *K. intermedius* V-05 in optimized and standard medium.

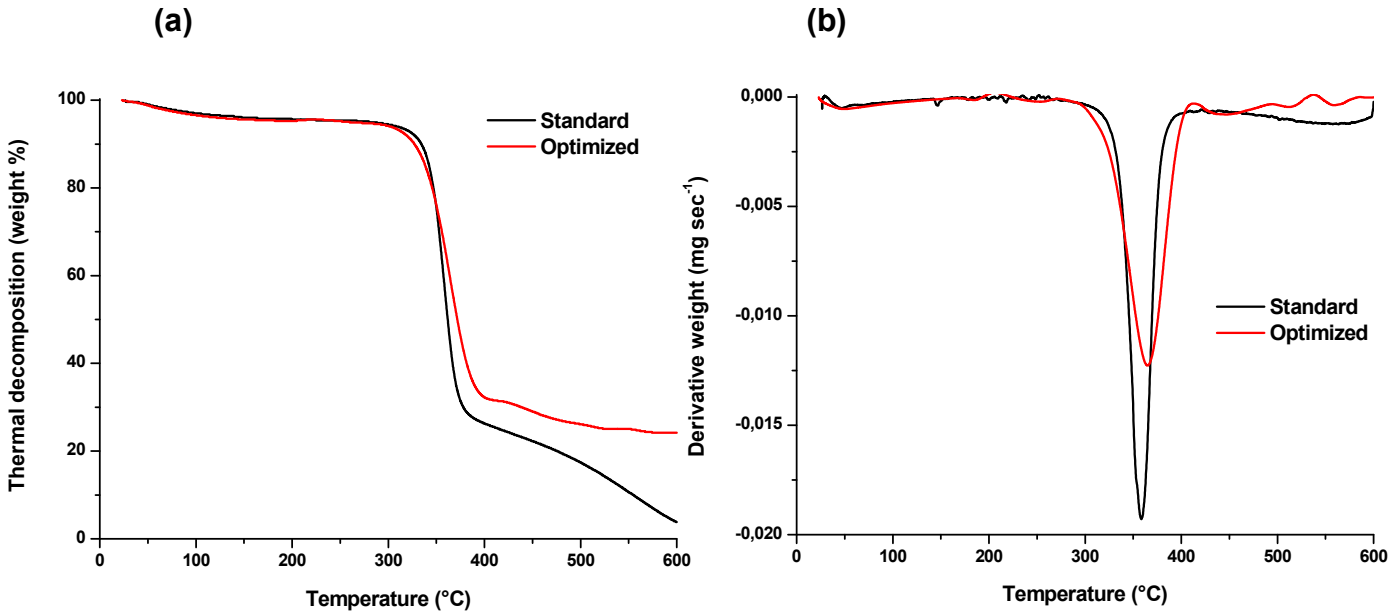


4.3.3.7. TG and DTG analysis

Thermal decomposition and the respective DTG curves of BC membranes are shown in Figure 4.

The samples produced in both optimized and standard medium presented three distinct mass-loss events, which are characteristics of pure BC. The first event was observed from room temperature (approximately 30 ºC) to approximately 150 ºC and attributed to mass loss due to evaporation of residual water from the polymeric matrix. The second event was observed between the temperature range from 300 to 400 ºC and attributed to degradation of cellulose due to dehydration and decomposition of the glycosidic units. Finally, the third event occurred up to 600 ºC corresponding to the thermo-oxidative degradation (pyrolysis) of cellulose (VASCONCELOS et al., 2017; SALVI et al., 2014).

Figure 4. TGA **(a)** and DTG **(b)** curves of bacterial cellulose produced by *K. intermedius* V-05 in optimized standard and media.



From the plotted curves were determined the temperatures of initial thermal decomposition (T_{onset}), final thermal decomposition (T_{endset}), and maximum weight loss rate (T_{max}), as shown in Table 3. It was observed that production in optimized medium slightly increased the temperatures of maximum weight loss and final thermal degradation compared to the production in HS medium. These facts demonstrated a higher energy necessary for degradation of membranes produced from optimized medium. The profiles of thermal stability and degradation temperature of BC observed in this work were similar to those observed by Souza et al. (2020).

The values of T_{onset} were similar to the reported by Rojo et al. (2021) and Fu et al. (2021) in which they found values around 330 ºC. The maximum weight loss occurred between 300 and 400 ºC (Table 5) indicating that the samples were stable up to this temperature, similarly to reported in other studies of thermal characterization of BC (MOLINA-RAMÍREZ et al., 2020; LIN et al., 2016). The improvement in thermal stability makes BC produced in this work a very attractive raw material for food packaging applications.

Table 5. Weight loss by temperature range of cellulose membranes produced by *K. intermedius* V-05 in optimized and standard media.

Temperature range	Weight loss (%)	
	Optimized	**Standard**
30-100°C	3.0	3.0
100-200°C	1.4	1.4
200-300°C	1.1	1.2
300-400°C	62.3	68.3
400-500°C	6.0	8.8
500-600°C	2.0	13.5

4.3.3.8. Hydrophilic properties

The characterization of the BC membranes regarding water holding capacity (WHC) and rehydration capacity (RC) is shown in Table 3. All the samples had moisture content superior to 99.0% (w/w).

Membranes produced in optimized medium exhibited higher WHC and RC than those produced in HS medium. Both parameters have a direct relation with the porosity and surface area of the BC matrix, which means that a high WHC and RC indicate a more porous membrane (UL-ISLAM; KHAN; PARK, 2012). While WHC is related to never-dried BC, RC represents the degree to which removed water was recovered by the samples. Drying process improves the storage and sell-life of BC, but poor rehydration capacity reduces the utility of dried BC (HUANG et al., 2010; LIN et al., 2009).

Because membranes obtained from optimized media presented lower dry weight and crystallinity compared to those from standard medium, the observed increase in hydrophilic properties may be correlated to the increase of empty spaces among BC fibrils caused by the lower density of these membranes. These facts caused an increase in porosity and provided more space for water accommodation. Consequently, more water could penetrate and adsorb onto the material (UL-ISLAM; KHAN; PARK, 2012). The high water holding capacity makes BC an excellent wound healing material by maintaining a moist environment and helping to eliminate exudates (ESLAHI et al., 2020).

The WHC values of BC generally vary between 100 and 200 times its dry weight. However, values reaching up to 400 times dry weight were described by Mohite and Patil (2014). The values observed in this work were similar to those observed by Bandyopadhyay et al. (2018) for BC produced from waste apple juice in which they achieved values between 100 and 250 times dry weight.

4.3.4. Conclusion

The Plackett and Burman factorial design followed by response surface methodology were effective in selecting and determining the best concentration of required amino acids that affect BC production by the strain *K. intermedius* V-05. Also, the different amino acids had distinct effects on biosynthesis of BC. Aspartic acid, phenylalanine and serine presented more significant positive effect and improved production. Conversely, others like those containing sulfur or positively charged amino acids presented a significant negative effect and reduced the production yield.

The response surface methodology was a useful tool that made it possible to elaborate a synthetic growth medium capable of supplying essential amino acids required for biosynthesis of BC. Although optimized medium does not provide higher yield than the standard HS medium, its use could provide membranes with greater characteristics than those observed by using the complex medium, such as some thermal and hydrophilic properties.

Acknowledgements

We would like to thank “Laboratório de Espectroscopia (ESPEC)” and “Laboratório de Difração de Raios X (LabDRX)” of the State University of Londrina for providing equipments and assistance in the analyses of chemical characterization. The author also thanks “Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)” for providing scholarship during the study period (Process number: 142380/2017-2).

4.3.5. References

AGARWAL, P. K. Enzymes: An integrated view of structure, dynamics and function. **Microbial Cell Factories,** v. 5, n. 1, p. 1-12, 2006. https://doi.org/10.1186/1475-2859-5-2

BARUD, H.; RIBEIRO, C.; CRESPI, M.; MARTINES, M.; DEXPERT-GHYS, J.; MARQUES, R.; MESSADDEQ, Y.; RIBEIRO, S. Thermal characterization of bacterial cellulose–phosphate composite membranes. **Journal of Thermal Analysis and Calorimetry,** v. 87, n. 3, p. 815-818, mar. 2007. https://doi.org/10.1007/s10973-006-8170-5

BARUD, H. S.; ARAÚJO JÚNIOR, A. M.; SANTOS, D. B.; ASSUNÇÃO, R. M. N.; MEIRELES, C. S.; CERQUEIRA, D. A.; RODRIGUES FILHO, G.; RIBEIRO, C. A.; MESSADDEQ Y.; RIBEIRO, S. J. L. Thermal behavior of cellulose acetate produced from homogeneous acetylation of bacterial cellulose. **Thermochimica Acta,** v. 471, n. 1-2, p. 61-69, maio 2008. https://doi.org/10.1016/j.tca.2008.02.009

BANDYOPADHYAY, S.; SAHA, N.; SÁHA, P. Characterization of bacterial cellulose produced using media containing waste apple juice. **Applied Biochemistry and Microbiology,** v. 54, n. 6, p. 649-657, nov. 2018. https://doi.org/10.1134/S0003683818060042

BOUDRAHEM, F.; ZIANI, S.; AISSANI-BENISSAD, F. Application of response surface methodology based on central composite design for optimization of yellow bezacryl sorption on shoe soles waste. **Environmental Progress & Sustainable Energy,** v. 38, n. s1, p. S412-S421, mar. 2019. https://doi.org/10.1002/ep.13073

CHANDRASEKARAN, P. T.; BARI, N. K.; SINHA, S. Enhanced bacterial cellulose production from *Gluconobacter xylinus* using super optimal broth. **Cellulose,** v. 24, n. 10, p. 4367-4381, out. 2017. https://doi.org/10.1007/s10570-017-1419-2

DINARVAND, M.; REZAEE, M.; FOROUGHI, M. Optimizing culture conditions for production of intra and extracellular inulinase and invertase from *Aspergillus niger* ATCC 20611 by response surface methodology (RSM). **Brazilian Journal of Microbiology,** v. 48, n. 3, p. 427-441, jul./set. 2017. http://doi.org/10.1016/j.bjm.2016.10.026

DU, L.; LIU, S.; HAO, G.; ZHANG, L.; ZHOU, M.; BAO, Y.; DING, B.; SUN, Q.; ZHANG, G. Preparation and release profiles *in vitro/vivo* of galantamine pamoate loaded Poly (Lactideco-Glycolide)(PLGA) microspheres. **Frontiers in Pharmacology,** v. 11, p. 2392, mar. 2021. https://doi.org/10.3389/fphar.2020.619327

EL-SAYED, W. M. M.; IBRAHIM, H. A. H.; ABDUL-RAOUF, U. M.; EL-NAGAR, M. M. Evaluation of bioethanol production from Ulva lactuca by Saccharomyces cerevisiae. **Journal of Biotechnology & Biomaterials,** v. 6, n. 2, p. 1000226, 2016. http://doi.org/10.4172/2155-952X.1000226

EJAZ, U.; AHMED, A.; SOHAIL, M. Statistical optimization of immobilization of yeast cells on corncob for pectinase production. **Biocatalysis and Agricultural Biotechnology,** v. 14, p. 450-456, abr. 2018. https://doi.org/10.1016/j.bcab.2018.04.011

ESLAHI, N.; MAHMOODI, A.; MAHMOUDI, N.; ZANDI, N.; SIMCHI, A. Processing and properties of nanofibrous bacterial cellulose-containing polymer composites: A review of recent advances for biomedical applications. **Polymer Reviews,** v. 60, n. 1, p. 144-170, 2020. https://doi.org/10.1080/15583724.2019.1663210

FENG, X.; ULLAH, N.; WANG, X.; SUN, X.; LI, C.; BAI, Y.; CHEN, L.; LI, Z. Characterization of bacterial cellulose by *Gluconacetobacter hansenii* CGMCC 3917. **Journal of Food Science,** v. 80, n. 10, p. E2217-E2227, out. 2015. https://doi.org/10.1111/1750-3841.13010

FERREIRA, S. L.; CAIRES, A. O.; BORGES, T. D. S.; LIMA, A. M.; SILVA, L. O.; SANTOS, W. N. Robustness evaluation in analytical methods optimized using experimental designs. **Microchemical Journal,** v. 131, p. 163-169, mar. 2017. https://doi.org/10.1016/j.microc.2016.12.004

FU, R.; REN, Y.; FANG, K.; SUN, Y.; ZHANG, Z.; LUO, A. Preparation, Characterization and Biocompatibility of Chitosan/TEMPO-oxidized Bacterial Cellulose Composite Film for Potential Wound Dressing Applications. **Fibers and Polymers,** v. 22, p. 1790-1799, jul. 2021. https://doi.org/10.1007/s12221-021-0854-8

GOMES, R. J.; FARIA-TISCHER, P. C. S.; TISCHER, C. A., CONSTANTINO, L. V.; ROSA, M. F.; CHIDEROLI, R. T.; PEREIRA, U. P.; SPINOSA, W. A. *Komagataeibacter intermedius* V-05: an acetic acid bacterium isolated from vinegar

industry, with high capacity for bacterial cellulose production in soybean molasses medium. **Food Technology and Biotechnology,** v. 59, n. 4, out./dez. 2021. https://doi.org/10.17113/ftb.59.04.21.7148

GRAHOVAC, J.; GRAHOVAC, M.; DODIĆ, J.; BAJIĆ, B.; BALAŽ, J. Optimization of cultivation medium for enhanced production of antifungal metabolites by *Streptomyces hygroscopicus*. **Crop Protection,** v. 65, p. 143-152, nov. 2014. https://doi.org/10.1016/j.cropro.2014.07.020

GÜZEL, M.; AKPINAR, Ö. Preparation and characterization of bacterial cellulose produced from fruit and vegetable peels by *Komagataeibacter hansenii* GA2016. **International Journal of Biological Macromolecules,** v. 162, p. 1597-1604, nov. 2020. https://doi.org/10.1016/j.ijbiomac.2020.08.049

HESTRIN, S.; SCHRAMM, M. Synthesis of cellulose by *Acetobacter xylinum*. 2. Preparation of freeze-dried cells capable of polymerizing glucose to cellulose. **Biochemistry Journal,** v. 58, n. 2, p. 345-352, out. 1954.

HEO, M.-S.; SON, H.-J. Development of an optimized, simple chemically defined medium for bacterial cellulose production by Acetobacter sp. A9 in shaking cultures. **Biotechnology and Applied Biochemistry,** v. 36, n. 1, p. 41-45, ago. 2002. https://doi.org/10.1042/BA20020018

HUANG, H.-C.; CHEN, L.-C.; LIN, S.-B.; HSU, C.-P.; CHEN, H.-H. In situ modification of bacterial cellulose network structure by adding interfering substances during fermentation. **Bioresource Technology,** v. 101, n. 15, p. 6084-6091, ago. 2010. https://doi.org/10.1016/j.biortech.2010.03.031

ISLAM, M. U.; ULLAH, M. W.; KHAN, S.; SHAH, N.; PARK, J. K. Strategies for cost-effective and enhanced production of bacterial cellulose. **International Journal of Biological Macromolecules,** v. 102, p. 1166-1173, set. 2017. https://doi.org/10.1016/j.ijbiomac.2017.04.110

JAWAN, R.; ABBASILIASI, S.; TAN, J. S.; MUSTAFA, S.; HALIM, M.; ARIFF, A. B. Influence of culture conditions and medium compositions on the production of bacteriocin-like inhibitory substances by *Lactococcus lactis* Gh1. **Microorganisms,** v. 8, n. 10, p. 1454, 2020. https://doi.org/10.3390/microorganisms8101454

KAČURÁKOVÁ, M.; SMITH, A. C.; GIDLEY, M. J.; WILSON, R. H. Molecular interactions in bacterial cellulose composites studied by 1D FT-IR and dynamic 2D FT-IR spectroscopy. **Carbohydrate Research,** v. 337, n. 12, p. 1145-1153, 2002.

KESHK, S. M. A. S. Vitamin C enhances bacterial cellulose production in *Gluconacetobacter xylinus*. **Carbohydrate polymers,** v. 99, p. 98-100, jan. 2014. https://doi.org/10.1016/j.carbpol.2013.08.060

KOHLMEIER, Martin. **Nutrient metabolism: structures, functions, and genes.** 2 ed. Cambridge: Academic Press, 2015.

KONDO, T. The assigment of IR absorption bands due to free hidroxyl groups in cellulose. **Cellulose,** v. 4, p. 281-292, dez. 1997.

KUMAR, S.; PAKSHIRAJAN, K.; DASU, V. V. Development of medium for enhanced production of glutaminase-free L-asparaginase from *Pectobacterium carotovorum* MTCC 1428. **Applied Microbiology and Biotechnology,** v. 84, n. 3, p. 477-486, set. 2009. https://doi.org/10.1007/s00253-009-1973-0

LEONARDI, R.; JACKOWSKI, S. Biosynthesis of pantothenic acid and coenzyme A. **EcoSal Plus,** v. 2, n. 2, abr. 2007. https://doi.org/10.1128/ecosalplus.3.6.3.4

LI, X.; LI, J.; GONG, J.; KUANG, Y.; MO, L.; SONG, T. Cellulose nanocrystals (CNCs) with different crystalline allomorph for oil in water Pickering emulsions. **Carbohydrate Polymers,** v. 183, p. 303-310, mar. 2018. https://doi.org/10.1016/j.carbpol.2017.12.085

LIN, S. B.; HSU, C. P.; CHEN, L. C.; CHEN, H. H. Adding enzymatically modified gelatin to enhance the rehydration abilities and mechanical properties of bacterial cellulose. **Food Hydrocolloids,** v. 23, n. 8, p. 2195-2203, dez. 2009. https://doi.org/10.1016/j.foodhyd.2009.05.011

LIN, S. P.; LIU, C. T.; HSU, K. D.; HUNG, Y. T.; SHIH, T. Y.; CHENG, K. C. Production of bacterial cellulose with various additives in a PCS rotating disk bioreactor and its material property analysis. **Cellulose,** v. 23, n. 1, p. 367-377, fev. 2016. https://doi.org/10.1007/s10570-015-0855-0

LIN, D.; LIU, Z.; SHEN, R.; CHEN, S.; YANG, X. Bacterial cellulose in food industry: Current research and future prospects. **International Journal of Biological Macromolecules,** v. 158, p. 1007-1019, set. 2020. https://doi.org/10.1016/j.ijbiomac.2020.04.230

LUTHRA, U.; SINGH, N.; TRIPATHI, A.; VORA, S.; BHOSLE, V. Media optimization for lovastatin production by statistical approach using *Aspergillus terreus* by submerged fermentation. **Journal of Medical Science And clinical Research,** v. 3, n. 2, p. 4520-4528, fev. 2015.

MATSUOKA, M.; TSUCHIDA, T.; MATSUSHITA, K.; ADACHI, O.; YOSHINAGA, F. A synthetic medium for bacterial cellulose production by *Acetobacter xylinum* subsp. *sucrofermentans*. **Bioscience, Biotechnology, and Biochemistry,** v. 60, n. 4, p. 575-579, jan. 1996. https://doi.org/10.1271/bbb.60.575

MOHAMMADI, R.; MOHAMMADIFAR, M. A.; MORTAZAVIAN, A. M.; ROUHI, M.; GHASEMI, J. B.; DELSHADIAN, Z. Extraction optimization of pepsin-soluble collagen from eggshell membrane by response surface methodology (RSM). **Food Chemistry,** v. 190, p. 186-193, jan. 2016. https://doi.org/10.1016/j.foodchem.2015.05.073

MOHITE, B. V.; PATIL, S. V. Physical, structural, mechanical and thermal characterization of bacterial cellulose by *G. hansenii* NCIM 2529. **Carbohydrate Polymers,** v. 106, p. 132-141, jun. 2014. https://doi.org/10.1016/j.carbpol.2014.02.012

MOLINA-RAMÍREZ, C.; CAÑAS-GUTIÉRREZ, A.; CASTRO, C.; ZULUAGA, R.; GAÑÁN, P. Effect of production process scale-up on the characteristics and properties of bacterial nanocellulose obtained from overripe Banana culture medium. **Carbohydrate Polymers,** v. 240, p. 116341, jul. 2020. https://doi.org/10.1016/j.carbpol.2020.116341

MYERS, R. H.; KHURI, A. I.; CARTER, W. H. Response surface methodology: 1966–l988. **Technometrics,** v. 31, n. 2, p. 137-157, maio 1989. http://doi.org/10.1080/00401706.1989.10488509

NÚÑEZ, D.; CÁCERES, R.; IDE, W.; VARAPRASAD, K.; OYARZÚN, P. An ecofriendly nanocomposite of bacterial cellulose and hydroxyapatite efficiently removes lead from water. **International Journal of Biological Macromolecules,** v. 165, p. 2711-2720, dez. 2020. https://doi.org/10.1016/j.ijbiomac.2020.10.055

NURFARAHIN, A. H.; MOHAMED, M. S.; PHANG, L. Y. Culture medium development for microbial-derived surfactants production—an overview. **Molecules,** v. 23, n. 5, p. 1049, 2018. https://doi.org/10.3390/molecules23051049

PEREIRA, L.; GIRARDI, J. P.; BAKOVIC, M. Forms, crosstalks, and the role of phospholipid biosynthesis in autophagy. **International Journal of Cell Biology,** v. 2012, p. 931956, jan. 2012. https://doi.org/10.1155/2012/931956

POGORELOVA, N.; ROGACHEV, E.; DIGEL, I.; CHERNIGOVA, S.; NARDIN, D. Bacterial cellulose nanocomposites: Morphology and mechanical properties. **Materials,** v. 13, n. 12, p. 2849, 2020. https://doi.org/10.3390/ma13122849

QIU, J.; SONG, F.; QIU, Y.; LI, X.; GUAN, X. Optimization of the medium composition of a biphasic production system for mycelial growth and spore production of *Aschersonia placenta* using response surface methodology. **Journal of Invertebrate Pathology,** v. 112, n. 2, p. 108-115, fev. 2013. https://doi.org/10.1016/j.jip.2012.10.010

RAJPUT, K. N.; PATEL, K. C.; TRIVEDI, U. B. Screening and selection of medium components for cyclodextrin glucanotransferase production by new alkaliphile Microbacterium terrae KNR 9 using plackett-burman design. **Biotechnology Research International,** v. 2016, p. 3584807, 2016. http://doi.org/10.1155/2016/3584807

ROJO, Ú. M.; ROSSI, E.; CERRUTTI, P.; ERREA, M. I.; FORESTI, M. L. Preparation of water insoluble carboxymethylated bacterial cellulose with maximum lead retention capacity. **Journal of Polymer Research,** v. 28, n. 6, p. 1-7, 2021. https://doi.org/10.1007/s10965-021-02565-3

SAHM, F.; OEZEN, I.; OPITZ, C. A.; RADLWIMMER, B.; VON DEIMLING, A.; AHRENDT, T.; ADAMS S.; BODE, H. B.; GUILLEMIN, G. J.; WICK, W.; PLATTEN, M. The endogenous tryptophan metabolite and NAD+ precursor quinolinic acid confers resistance of gliomas to oxidative stress. **Cancer Research,** v. 73, n. 11, p. 3225-3234, jun. 2013. https://doi.org/10.1158/0008-5472

SALVI, D. T.; BARUD, H. D. S.; TREU-FILHO, O.; PAWLICKA, A.; MATTOS, R. I.; RAPHAEL, E.; RIBEIRO, S. J. Preparation, thermal characterization, and DFT study of the bacterial cellulose. **Journal of Thermal Analysis and Calorimetry,** v. 118, n. 1, p. 205-215, out. 2014. https://doi.org/10.1007/s10973-014-3969-y

SEGAL, L. G. J. M. A.; CREELY, J. J.; MARTIN JR, A. E.; CONRAD, C. M. An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer. **Textile Research Journal,** v. 29, n. 10, p. 786-794, out. 1959. https://doi.org/10.1177/004051755902901003

SEMJONOVS, P.; RUKLISHA, M.; PAEGLE, L.; SAKA, M.; TREIMANE, R.; SKUTE, M.; ROZENBERGA, L.; VIKELE, L.; SABOVICS, M.; CLEENWERCK, I. Cellulose synthesis by *Komagataeibacter rhaeticus* strain P 1463 isolated from Kombucha. **Applied Microbiology and Biotechnology,** v. 101, n. 3, p. 1003-1012, fev. 2017. https://doi.org/10.1007/s00253-016-7761-8

SINGH, V.; HAQUE, S.; NIWAS, R.; SRIVASTAVA, A.; PASUPULETI, M.; TRIPATHI, C. K. M. Strategies for fermentation medium optimization: an in-depth review. **Frontiers in Microbiology,** v. 7, p. 2087, jan. 2017. https://doi.org/10.3389/fmicb.2016.02087

SOMMER, A.; STAROSZCZYK, H.; SINKIEWICZ, I.; BRUŹDZIAK, P. Preparation and characterization of films based on disintegrated bacterial cellulose and montmorillonite. **Journal of Polymers and the Environment,** v. 29, n. 5, p. 1526-1541, maio 2021. https://doi.org/10.1007/s10924-020-01968-5

SON, H.-J.; KIM, H.-G.; KIM, K.-K.; KIM, H.-S.; KIM, Y.-G.; LEE, S.-J. Increased production of bacterial cellulose by *Acetobacter* sp. V6 in synthetic media under shaking culture conditions. **Bioresource Technology,** v. 86, n. 3, p. 215-219, fev. 2003. https://doi.org/10.1016/S0960-8524(02)00176-1

SOUZA, E. F.; FURTADO, M. R.; CARVALHO, C. W.; FREITAS-SILVA, O.; GOTTSCHALK, L. M. Production and characterization of *Gluconacetobacter xylinus* bacterial cellulose using cashew apple juice and soybean molasses. **International Journal of Biological Macromolecules,** v. 146, p. 285-289, mar. 2020. https://doi.org/10.1016/j.ijbiomac.2019.12.180

SPRY, C.; KIRK, K.; SALIBA, K. J. Coenzyme A biosynthesis: an antimicrobial drug target. **FEMS Microbiology Reviews,** v. 32, n. 1, p. 56-106, jan. 2008. https://doi.org/10.1111/j.1574-6976.2007.00093.x

SUGIYAMA, J.; PERSSON, J.; CHANZY, H. Combined infrared and electron diffraction study of the polymorphism of native celluloses. **Macromolecules,** v. 24, n. 9, p. 2461-2466, abr. 1991.

TEIXEIRA, S. R. Z.; REIS, E. M. D.; APATI, G. P.; MEIER, M. M.; NOGUEIRA, A. L.; GARCIA, M. C. F.; SCHNEIDER, A. L. S.; PEZZIN, A. P. T.; PORTO, L. M. Biosynthesis and Functionalization of Bacterial Cellulose Membranes with Cerium Nitrate and Silver Nanoparticles. **Materials Research,** v. 22, p. e20190054, 2019. https://doi.org/10.1590/1980-5373-MR-2019-0054

TORTORA, G. J.; FUNKE, B. R.; CASE, C. L. **Microbiologia.** 12 ed. Porto Alegre: Artmed, 2017.

UL-ISLAM, M.; KHAN, T.; PARK, J. K. Water holding and release properties of bacterial cellulose obtained by in situ and ex situ modification. **Carbohydrate Polymers,** v. 88, n. 2, p. 596-603, abr. 2012. https://doi.org/10.1016/j.carbpol.2012.01.006

VANLINDEN, M. R.; SKOGE, R. H.; ZIEGLER, M. Discovery, metabolism and functions of NAD and NADP. **The Biochemist,** v. 37, n. 1, p. 9-13, fev. 2015. https://doi.org/10.1042/BIO03701009

VASCONCELOS, N. F.; FEITOSA, J. P. A.; GAMA, F. M. P.; MORAIS, J. P. S.; ANDRADE, F. K.; SOUZA FILHO, M. S. M.; ROSA, M. F. Bacterial cellulose nanocrystals produced under different hydrolysis conditions: Properties and morphological features. **Carbohydrate Polymers,** v. 155, p. 425-431, jan. 2017. https://doi.org/10.1016/j.carbpol.2016.08.090

WANG, J.; TAVAKOLI, J.; TANG, Y. Bacterial cellulose production, properties and applications with different culture methods–A review. **Carbohydrate Polymers,** v. 219, p. 63-76, set. 2019. https://doi.org/10.1016/j.carbpol.2019.05.008

WANG, J.; WAN, W. Experimental design methods for fermentative hydrogen production: A review. **International Journal of Hydrogen Energy,** v. 34, n. 1, p. 235-244, jan. 2009. https://doi.org/10.1016/j.ijhydene.2008.10.008

WU, Guoyao. Amino acids: metabolism, functions, and nutrition. **Amino acids,** v. 37, n. 1, p. 1-17, 2009. https://doi.org/10.1007/s00726-009-0269-0

YIM, S. M.; SONG, J. E.; KIM, H. R. Production and characterization of bacterial cellulose fabrics by nitrogen sources of tea and carbon sources of sugar. **Process Biochemistry,** v. 59, p. 26-36, ago. 2017. https://doi.org/10.1016/j.procbio.2016.07.001

YOLMEH, M.; JAFARI, S. M. Applications of response surface methodology in the food industry processes. **Food and Bioprocess Technology,** v. 10, n. 3, p. 413-433, jan. 2017. https://doi.org/10.1007/s11947-016-1855-2

ZHANG, Q.; LI, Y.; XU, F.; ZHENG, M.; XI, X.; ZHANG, X.; HAN, C. Optimization of submerged fermentation medium for matrine production by *Aspergillus terreus*, an endophytic fungus harboring seeds of *Sophora flavescens*, using response surface methodology. **Mycobiology,** v. 45, n. 2, p. 90-96, 2017. https://doi.org/10.5941/MYCO.2017.45.2.90

ZHU, F.; PEÑA, M.; BENNETT, G. N. Metabolic engineering of *Escherichia coli* for quinolinic acid production by assembling L-aspartate oxidase and quinolinate synthase as an enzyme complex. **Metabolic Engineering,** v. 67, p. 164-172, set. 2021. https://doi.org/10.1016/j.ymben.2021.06.007

CAPÍTULO IV – LONG PRE-CULTIVATION TIME BEFORE BACTERIAL CELLULOSE PRODUCTION IMPROVES THE CAPACITY OF BIOSYNTHESIS BY A LOW-PRODUCING STRAIN *KOMAGATAEIBACTER HANSENII*

ABSTRACT

Bacterial cellulose (BC) is an extracellular polysaccharide that has several applications in medical, pharmaceutical and food industry mainly due to its greater properties compared to plant-origin cellulose. BC is principally synthetized by acetic acid bacteria (AAB) of the genus *Komagataeibacter* through fermentative process. However, high costs and low production yield make difficult its large-scale application. The aim of this work was to evaluate the effects of pre-cultivation time before fermentation on the ability to increase biosynthesis capacity of BC by low-producing strain. Then, produced BC were characterized by X-Ray diffraction (XRD), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), infrared spectroscopy (FT-IR) and hydrophilic properties. The strain *Komagataeibacter hansenii* ATCC 23769 was cultivated in fermentation broth for two periods of 35 or 56 days under static conditions. At the end of each period of time, they were transferred to new broth to be cultivated again (new cycle) for 35 or 56 days and carried out in parallel a 10-day fermentation to determine the quantity of BC produced. As result, a greater increase was observed after the end of the second and third pre-cultivation period of 56 days (increase of 137% and 187% in relation to non-cultivated strain, respectively). This simple method could spontaneously select most productive strains and improve the capacity of BC biosynthesis being influenced by time and number of cycles before fermentation. Produced samples presented higher crystallinity and thermal properties but lower water holding capacity. Through this work, it was observed that the longer time of pre-cultivation, the greater increase in capacity of cellulose biosynthesis also depending on the number of cultivation cycles carried out.

Key words: microorganism; fermentation; method development; biopolymer.

4.4.1. Introduction

Acetic acid bacteria (AAB) comprise a group of microorganisms commonly recognized and utilized for their strong ethanol-oxidizing capacity as well as their high resistance to the acetic acid released into the fermentation medium. They are widely spread in nature and in several regions of the world which demonstrate their great potential of adaptation. AAB can play an important role in production of foods and beverages, such as vinegar, kombucha beverage, and cocoa, but they can also cause food spoilage, such as in wine, beer, and fruits (QIU; ZHANG; HONG, 2021; SHARAFI; RASOOLI; BEHESHTI-MAAL, 2010; SENGUN; KARABIYIKLI, 2011; STASIAK; BŁAŻEJAK, 2009).

AAB are strictly aerobic microorganisms, Gram-negative or Gram-variable, catalase-positive and oxidase-negative, ellipsoidal and rod-shaped cells that can occur singly, in pairs or chains. They are also mesophilic microorganisms able to growth in temperatures between 25 and 30 ºC. AAB strains are well-known to have a high capability to oxidise alcohols, aldehydes, sugars or sugar alcohols in the presence of oxygen (GOMES et al., 2018).

In addition to produce acetic acid as the main active component of vinegar, some strains are also able to produce other important biomolecules, such as dihydroxyacetone from glycerol (STASIAK-RÓŻAŃSKA; BERTHOLD-PLUTA; DIKSHIT, 2018); sorbose from sorbitol by the genus *Gluconobacter* (XU et al., 2014), which is the first step in the industrial production of ascorbic acid; and cellulose by the genera *Gluconacetobacter* and *Komagataeibacter* (CACICEDO et al., 2016).

Bacterial cellulose (BC) production is easily detected on the surface of growth medium by the formation of a gelatinous biofilm. This substance also denominated as “mother of vinegar” has the same structure, composition and reactivity that plant-origin cellulose. It has been suggested that the cellulosic biofilm produced by AAB has the function of anchoring the cells on the surface and allows the bacteria to remain at the air/liquid interface to get the necessary oxygen for their metabolism. Additionally, the microbial biofilm can form a barrier to protect cells against ultraviolet radiation; it increases the ability to colonize other substrates due to its hygroscopic nature; and allows microorganisms to retain moisture and prevent dehydration (CACICEDO et al., 2016).

Cellulose can be synthesized by many different organisms, including plants, algae and some bacteria. However, due to specific properties and characteristics of BC, the production through microorganisms has attracted interest over the years. Unlike plant-origin cellulose, BC is a hemicellulose and lignin-free fiber, which shows superior physicochemical and mechanical properties, such as: higher purity, higher crystallinity, higher degree of polymerisation, high water-absorbing and -holding capacities, higher tensile strength, and excellent biocompatibility and biodegradability. In addition to these properties, BC is non-toxic (GOMES et al., 2018; MOHAMMADKAZEMI; AZIN; ASHORI, 2015). Despite the several advantages, it is still difficult to obtain high productivity mainly in large-scale fermentation systems due to low yield of BC production, especially in agitated cultivation method (LIU et al., 2018). In static cultivation systems, high costs and low production rate are also the main problems reported (WANG; TAVAKOLI; TANG, 2017). Thus, it is relevant and necessary to develop methods to produce bacterial cellulose with high productivity and low production costs.

Enhancement of microbial metabolite productivity by microorganisms can be done by manipulating nutritional parameters, environmental conditions and strain improvement (SARRAFZADEH, 2012). However, it has still been a challenge to increase capacity of biosynthesis BC in producing bacterial strains by using genetic engineering techniques. Because of this, researches focused on reducing the costs of BC synthesis have been carried out mainly based on the isolation of more productive bacterial strains, optimization of cultivation conditions and use of low-cost substrates (SEMJONOVS et al., 2017).

In some reports, strain improvement was achieved by limiting the ability for gluconic acid production in a *K. xylinus* mutant since this acid leads to a decrease in pH of the medium and induces to a reduction in BC production. By gene disrupting of glucose dehydrogenase (GDH), a GDH-deficient mutant strain which does not produce gluconic acid was able to produce the double of BC as compared to wild strain (SHIGEMATSU et al., 2005; ULLAH et al., 2016). Mutant strains were also generated by using ultraviolet (UV) radiation followed by ethyl methane sulfonate (EMS) treatment (HUNGUND; GUPTA, 2010). In another study, improvement of BC yield was achieved in a *K. xylinus* mutant produced by using high hydrostatic pressure (WU et al., 2010).

Considering the importance of studies to obtain BC with better yield, this work aimed to evaluate the effects of pre-cultivation for long period of time before fermentative production on the ability to increase capacity of biosynthesis by a low-producing *Komagataeibacter* strain. BC membranes were characterized by X-Ray diffraction (XRD), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and hydrophilic properties to determine their characteristics.

4.4.2. Material and Methods

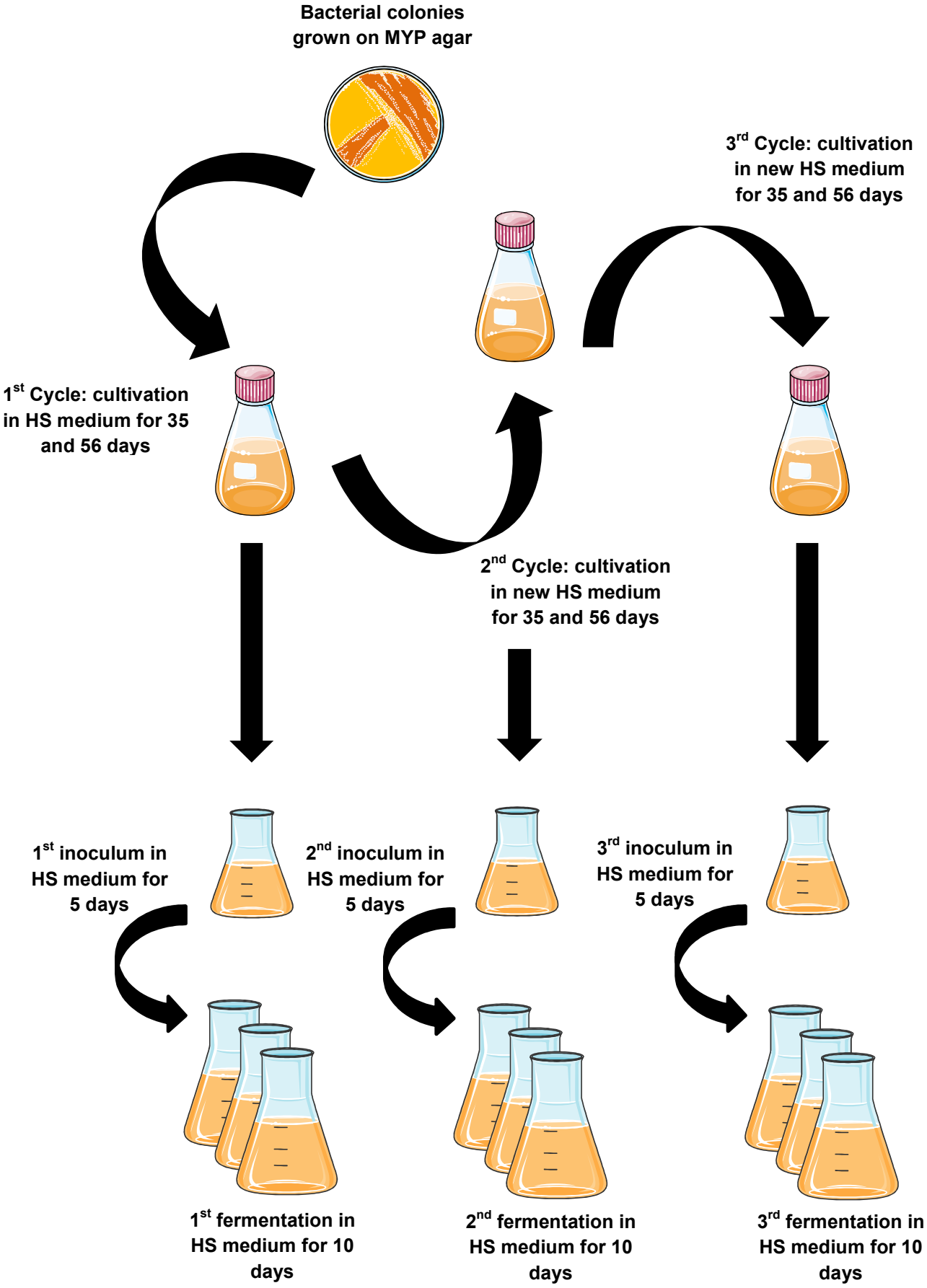
4.4.2.1. Material

In this work, it was used an AAB named *Komagataeibacter hansenii* ATCC 23769 that was acquired from collection culture (Embrapa Tropical Agroindustry, Fortaleza, Brazil). Cellulase enzyme (Celumax C®) was obtained from Prozyn (São Paulo, SP, Brazil). All reagents and chemicals used were of analytical grade and purchased from different companies, such as: Anidrol (Diadema, SP, Brazil), Himedia (Mumbai, India), Nuclear (Diadema, SP, Brazil), Synth (Diadema, SP, Brazil) and Vetec Química (Rio de Janeiro, RJ, Brazil).

4.4.2.2. Activation of microorganisms, inoculum an fermentation process

Figure 1 schematizes the general procedure for the first three cultivation cycles that were used to increase the capacity of BC biosynthesis by the strain. Initially, bacterial colonies were grown for 96h on mannitol–yeast extract–peptone (MYP) agar composed of 25 g L^{-1} mannitol, 5 g L^{-1} yeast extract, 3 g L^{-1} peptone and 10 g L^{-1} agar. Then, these colonies were transferred into flasks containing 100 mL of Hestrin and Schramm (HS) medium composed of 20 g L^{-1} glucose, 5 g L^{-1} peptone, 5 g L^{-1} yeast extract, 2.7 g L^{-1} Na_2HPO_4 and 1.15 g L^{-1} citric acid. The flasks were kept in microbiological incubator (Tecnal TE-391) under static conditions for two different periods: (i) 35 days (or five weeks) and (ii) 56 days (or eight weeks) that comprised the first cycle of pre-cultivation. At the end of time period, a 1/10 fraction of the broth was transferred into new flasks containing 90 mL sterile HS medium to be cultivated again for a new cycle of 35 or 56 days, which comprised the second cycle of pre-cultivation. This procedure was sequentially performed for the other cycles.

Figure 1. Schematical design showing the long-time pre-cultivation cycles and sequential fermentation by *K. hansenii* ATCC 23769 used in the experiments.



Parallel to the transfer to new cycle, it was determined the capacity of BC biosynthesis by the cultivated strain at the end of each pre-cultivation time of 35 or 56 days. For this, a 1/10 fraction of the inoculated broth was transferred into 250 mL Erlenmeyer containing 50 mL sterile HS medium, which was kept under static conditions for 5 days to be used as an inoculum. Then, BC formed on the surface was hydrolyzed by using 0.02% cellulase enzyme (1-2 days at 30 ± 0.5 °C). After centrifugation (Eppendorf Centrifuge 5804R, Germany) for 15 min, at 5000 rpm (5 °C), a cell suspension containing 10^8 colony-forming units (CFU) per mL was transferred to new 500 mL Erlenmeyer containing 100 mL sterile HS medium. The flasks were kept under static conditions for 10 days to be determined the capacity of BC production by the pre-cultivated strain from each pre-cultivation cycle.

4.4.2.3. Quantification of bacterial cellulose produced

Bacterial cellulose produced on the surface of each production medium were collected after 10 days and purified by heating in 2% (w/v) NaOH solution for 1 h at 80 ºC. Then, they were washed with distilled water until obtaining neutral pH (NÚÑEZ et al., 2020). The cellulosic membranes were dried until obtaining constant weight. The obtained dry weight was used to determine the mass of BC in g L^{-1}.

4.4.2.4. Effects of pre-cultivation time and cycles on the aspect of bacterial cellulose produced

The visual and macroscopic aspects of BC membranes obtained in productive fermentation that was executed at the end of each pre-cultivation cycle were recorded and compared by digital images taken using a digital camera (Olympus VG-120, Tokyo, Japan).

4.4.2.5. Characterization of bacterial cellulose produced

Because BC production after the end of 56-day pre-cultivation treatment present higher dry weight compared to those from the 35-day treatment, they were chosen to be characterized as follows:

4.4.2.5.1. Fourier transform infrared spectroscopy (FT-IR)

Infrared spectra were recorded using a spectrometer (Shimadzu, Prestige-21, Japan) over the 4000–400 cm^{-1} range. Dried samples produced from the 56-day pre-cultivation treatment were mixed with potassium bromide (KBr) and the respective spectra of were collected in transmission mode, with a resolution of 1 cm^{-1}.

4.4.2.5.2. X-ray diffraction (XRD)

The XRD patterns of BC membranes produced from the 56-day pre-cultivation treatment were obtained using a X-ray diffractometer (Malvern Panalytical X'Pert PRO MPD, Almelo, Netherlands) with Cu-Kα radiation (λ = 1.5418 Å) generated at 40 kV and 30 mA. The samples were analysed over the range of 5–70º (2θ) with an angular step of 0.04º. Counting time per point was 3 s. The degree of crystallinity for cellulose I was determined as follows (SEGAL et al., 1959):

$$\textbf{Crystallinity Index (\%)} = \frac{(I_{002} - Iam)}{I_{002}} \times 100$$

where I_{002} is the maximum peak intensity corresponding to the 002 lattice diffraction at angle 2θ = 22.8°; and **Iam** is the intensity of diffraction corresponding to the amorphous background at angle 2θ = 18°.

4.4.2.5.3. Differential scanning calorimetry (DSC)

DSC curves of BC samples produced from the 56-day pre-cultivation treatment were obtained using a Shimadzu DSC-60 (Tokyo, Japan) differential scanning calorimeter and indium as standard reference. The scans were started at room temperature and completed at 400 °C under a nitrogen atmosphere (50 mL min^{-1}) and heating rate of 10 °C min^{-1}.

4.4.2.5.4. Thermogravimetric analysis (TGA)

Thermal analysis of BC samples (approximately 5 mg) produced from the 56-day pre-cultivation treatment was performed in a Shimadzu TGA-50 (Tokyo, Japan) thermal analyser. The scans were ramped from room temperature to 600 ºC at a heating rate of 10 °C min^{-1}, under nitrogen atmosphere (50 mL min^{-1}).

The TGA curves were expressed as the mass variation as a function of temperature. Derivative form of TG curves (DTG) were expressed as the weight-loss rate as a function of temperature. From the plotted curves showing mass percentage as a function of temperature, were determined the parameters of initial (T_{onset}) and final (T_{endset}) thermal decomposition, maximum weight loss rate (T_{max}), 10% mass loss ($T_{10\%}$), and mass loss at 600 °C.

4.4.2.4.5. Hydrophilic properties

For determination of the water holding capacity (WHC), rehydration capacity and moisture content, never-dried BC samples produced from the 56-day pre-cultivation treatment were shaken quickly and weighed after being removed from the storage recipient (BC_{wet}). Then, the samples were dried until the water was completely removed, and weighed again (BC_{dry}). WHC was calculated by the following formula (FENG et al., 2015):

$$\textbf{Water holding capacity} = \frac{(BC_{wet} - BC_{dry})}{BC_{dry}}$$

The obtained dried samples (BC_{dry}) were immersed in distilled water until the weight of the rehydrated sample (BC_r) no longer increased. The reabsorption capacity was calculated by the following formula (TEIXEIRA et al., 2019):

$$\textbf{Rehydration capacity (\%)} = \frac{(BC_r - BC_{dry})}{BC_r} \times 100$$

The moisture content of the BC was obtained by using the following formula (CHANDRASEKARAN; BARI; SINHA, 2017):

$$\textbf{Moisture content (\%)} = \frac{(BC_{wet} - BC_{dry})}{BC_{wet}} \times 100$$

4.4.2.5. Statistical software and data analysis

Results of BC production reached in both 35-day and 56-day treatments were compared among each pre-cultivation cycle through Tukey test at 5% significance level by using the statistical software R® (R Core Team, Version 3.6.3).

4.4.3. Results and Discussion

4.4.3.1. Effects of the pre-cultivation time on the capacity of BC biosynthesis

The pre-cultivation of a low-producing AAB strain in a liquid culture medium for a long time of 35 and 56 improved the ability of maximum BC production (Table 1). This improvement was dependent on the pre-cultivation time and number cycles.

Table 1. Maximum capacity of BC production by K. *hansenii* ATCC 23769 reached after 10 days of fermentation at the end of each pre-cultivation cycle.

35-day pre-cultivation		56-day pre-cultivation	
Fermentation	**BC (g L^{-1})***	**Fermentation**	**BC (g L^{-1})***
1st cycle	$0.60^{c} \pm 0.03$	**1st cycle**	$0.86^{c} \pm 0.07$
2nd cycle	$0.70^{b} \pm 0.05$	**2nd cycle**	$1.42^{b} \pm 0.15$
3rd cycle	$1.13^{a} \pm 0.02$	**3rd cycle**	$1.72^{a} \pm 0.04$
4th cycle	$1.16^{a} \pm 0.05$	-	-
5th cycle	$1.14^{a} \pm 0.07$	-	-

* Means followed by equal letters in the same column do not differ significantly from each other based on the Tukey test ($p \leq 0.05$).

In the 35-day pre-cultivation treatment, it was not observed increase in mass of BC obtained in the productive fermentation carried out at the end of the 1st cycle, compared to those from the non-cultivated strain (0.60 g L^{-1}). However, there was an slight increase of 16.6% in mass of BC obtained in the productive fermentation carried out at the end of the 2nd cycle compared to those from the 1st cycle and non-cultivated strain. At the end of the 3rd, 4th and 5th cycles, the mass of BC obtained in the productive fermentation did not differed significantly ($p \leq 0.05$). Also, they represented an increase of 90.5% in mass of BC compared to those from the 1st cycle and non-cultivated strain. These results indicated that there was stabilization in the mass of BC produced by the strain after three cycles of pre-cultivation.

In the 56-day pre-cultivation treatment, it was observed a slight increase of 43.3% in mass of BC obtained in the productive fermentation carried out at the end of the 1st cycle, compared to those from the non-cultivated strain (0.60 g L^{-1}). At the end of the 2nd cycle, a higher increase of 65.1% and 137% was reached in the fermentative production compared to those from the 1st cycle and non-cultivated strain, respectively. At the end of the 3rd cycle, it was observed an increase of 100% and 187% compared to the mass of BC produced from the 1st cycle and non-cultivated strain, respectively. However, the observed increase in mass of BC produced from the 3rd cycle was 21.1% compared to those produced from the 2nd cycle. This result was lower than the increase observed in mass of BC produced from the 2nd cycle compared to the 1st cycle, which also may indicate stabilization in the capacity of BC biosynthesis after the three sequential cycles.

Two types of cells are present in bacteria of the genus *Komagataeibacter*. They are denominated as “Cel+”*,* which are able to produce cellulose, and as “Cel−”, which are cellulose-negative mutant cells (VALLA; KJOSBAKKEN, 1982). According to Krystynowicz et al. (2002), the different culture conditions (static or agitated) are the main factors that affect preferential selection of one or another type of cell. In agitated culture systems, the medium become more enriched with cellulose-negative mutants because they grow faster in aerated systems. In static fermentation systems, cellulose-synthesizing cells produce the exopolysaccharide on the surface of liquid-air interface where they can obtain oxygen. However, the gelatinous membrane can also limit the access of oxygen into lower depths of the cultivation medium (AHMED; GULTEKINOGLU; EDIRISINGHE, 2020; CAMPANO et al., 2016). Consequently, the

“Cel−” mutants die faster because they are mainly present in these parts of the liquid culture medium.

Comparing the two pre-cultivation times of 35 and 56 days (Table 1), it was observed that the longer time induced a greater increase in capacity of BC biosynthesis by the AAB strain. Possibly, after the end of each cycle a larger number of cells able to produce cellulose are present in the aged culture medium. This larger quantity of producing cells can grow faster in the new fermentation broth and produce more cellulose compared to non-cultivated strain. So, the longer time of pre-cultivation before productive fermentation can positively influence the selection of larger number of “Cel+” instead of “Cel−” mutants.

It has been reported that improvement of BC-producing strains was achieved by using high hydrostatic pressure treatment (double of BC production compared with the non-treated strain *K. xylinus*) (WU et al., 2010); by gene disruption of GDH (production 1.7-fold higher than the wild type *K. xylinus*) (SHIGEMATSU et al., 2005); and by using UV radiation (30% more BC production than the wild strain) and EMS mutagenic agent (98% more BC production than that of wild strain *K. xylinus*) (HUNGUND; GUPTA, 2010). Comparing these results with those from the present work, we have demonstrated that strain improvement for higher BC production could be achieved by a simpler, low-cost and easy-to-perform method.

The aspects of BC membranes produced by the strain *K. hansenii* ATCC 23769 after fermentation performed at the end of each pre-cultivation cycles can be seen in Figure 2. It was observed that longer time of pre-cultivation before productive fermentation provided more fibrous and thicker membranes, mainly for those from the 3rd cycle of pre-cultivation.

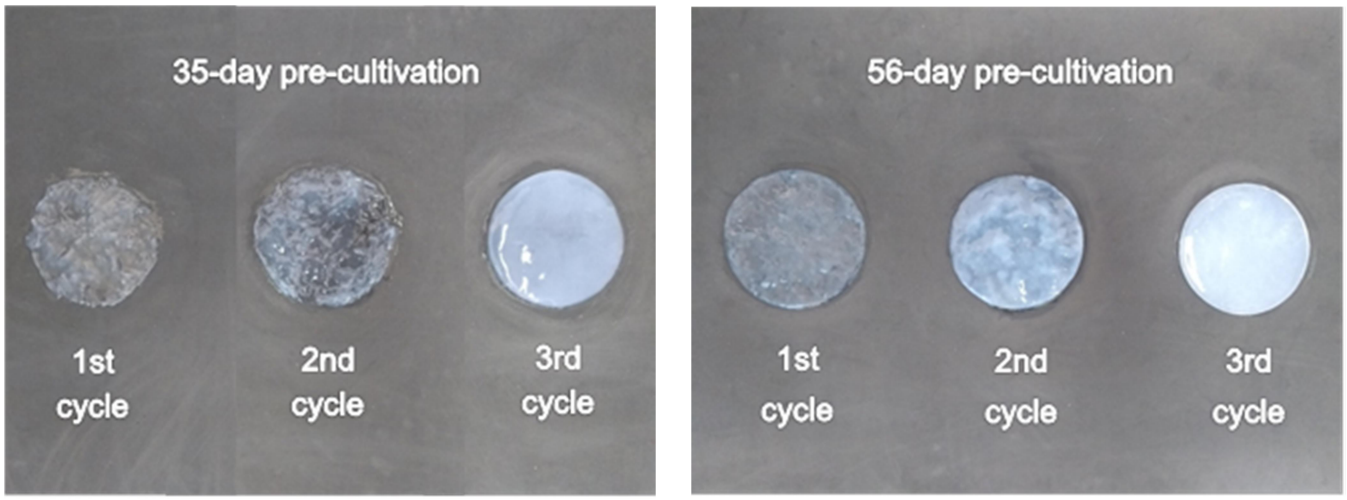
4.4.3.2. Characterization of bacterial cellulose produced

4.4.3.2.1. FT-IR analysis

BC spectra obtained by FT-IR spectroscopy are shown Figure 3(a). According to Kačuráková et al. (2002), Kondo (1997) and Sugiyama et al. (1991), the main band assignments that characterize the BC biopolymer, are: strong transmission of O-H stretching vibrations at 3400-3500 cm^{-1}; alkane C-H stretching and CH_2 asymmetric stretching vibration at 2900 cm^{-1}; CH_2 symmetric stretching vibration at

2700 cm^{-1}; O-H deformation vibration at 1600 cm^{-1}; CH_2 deformation vibration at 1400 cm^{-1}; CH_3 deformation vibration at 1370 cm^{-1}; C-O deformation vibration in the range of 1320-1030 cm^{-1}; and H-O-H bending vibration attributed to water absorption at 1640 cm^{-1}. These observed bands in the FT-IR spectra of BC membranes produced from the 56-day pre-cultivation treatment were described in Table 2 and were in accordance with previous studies of chemically pure bacterial cellulose (POGORELOVA et al., 2020). The absence of bands at 1735 cm^{-1} associated with functional groups present in proteins (C=O) and other bands of nitrogenous organic compounds mainly at 1538 cm^{-1} indicated that the purification process was efficient.

Figure 2. Aspect of BC membranes produced after 10-day fermentation at the end of each pre-cultivation cycles of 35 or 56 days by *K. hansenii* ATCC 23769 under static conditions.



4.4.3.2.2. XRD analysis

The analysed BC samples showed similar crystalline diffraction peaks in XRD spectra (Figure 3(b)). The two main peaks at 14.8º and 22.5º demonstrate that BC membranes presented typical crystalline forms of cellulose I. The crystallinity index of BC samples produced by the pre-cultivated strain (Table 3) varied between 63.5% (1st cycle) and 74.9% (3rd cycle), and they were higher than those from non-cultivated strain (60.7%, as shown in section 4.2.3.6.). These values of crystallinity index were similar to obtained by Fan et al. (2016) in BC produced from waste of citrus peel and pomace (65.0%) and by Chen et al. (2019) and Martínez-Sans et al. (2011) in BC produced from different carbon sources (61.0-78.0%).

Figure 3. FT-IR **(a)**, XRD **(b)** and DSC **(c)** spectra of the bacterial cellulose produced by *K. hansenii* ATCC 23769 at the end of the 56-day pre-cultivation cycles.

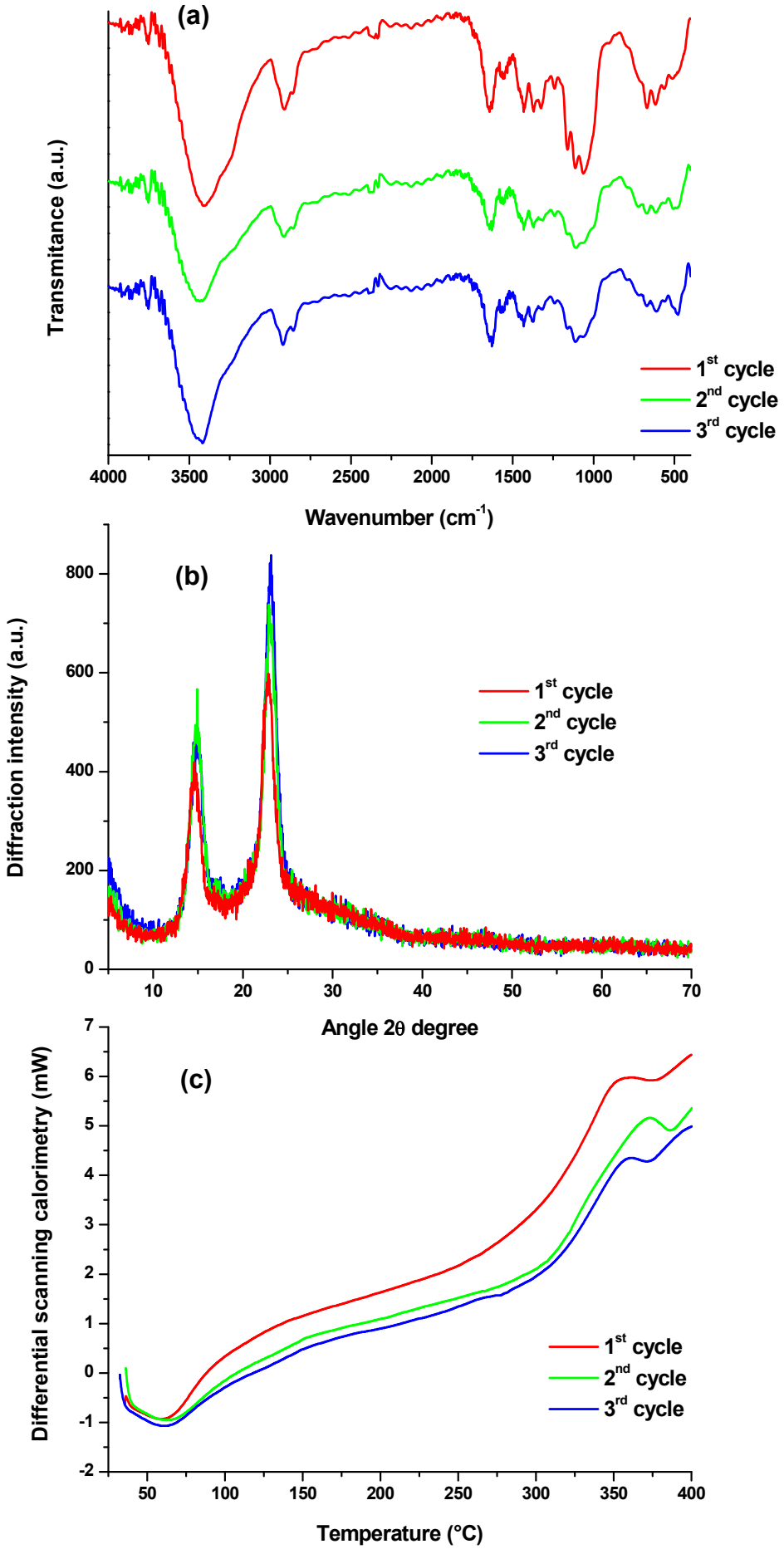


Table 2. Band assignments in the FT-IR spectra of cellulose membranes produced by *K. hansenii* ATCC 23769 at the end of the 56-day pre-cultivation cycles.

Band assignments	Wave number (cm^{-1})		
	1st cycle	**2nd cycle**	**3rd cycle**
O-H stretching vibration	3412	3437	3418
C-H stretching vibration	2912	2914	2920
O-H deformation vibration	1628	1628	1628
CH_2 deformation vibration	1431	1431	1431
CH_3 deformation vibration	1369	1369	1373
C-O deformation vibration	1113	1109	1113
H-O-H bending vibration of absorbed water	1649	1649	1649
Contribution from celulose Iα	n.d.	n.d.	n.d.
Contribution from celulose Iβ	n.d.	723	718

n.d. = not detected.

The increase in crystallinity index of the samples produced by the pre-cultivated strain may indicates an increase of crystalline regions and hydrophobic interactions, making these samples interesting sources for stable water/oil (W/O) emulsions preparation (LI et al., 2018). The two major diffraction peaks observed at 15.0º and 22.5º (Figure 3(b)) can be assigned to the interplane distances that are characteristic of cellulose Iα and Iβ phases corresponding to the primary diffraction of the crystal planes 100Iα, 110Iβ and 010Iβ planes at 15.0º and 110Iα and 200Iβ at 22.5º (MOHITE; PATIL, 2014). However, some authors have also suggested that the peak at 15.0º is mostly attributed to cellulose Iα and that at 22.5º is mostly attributed to cellulose Iβ (SOMMER et al., 2021; STANISŁAWSKA; STAROSZCZYK; SZKODO, 2020; VAZQUEZ et al., 2013).

4.4.3.2.3. DSC analysis

The heat absorbed or released by BC membranes as function of temperature was measured using DSC. The obtained patterns (Figure 3(c)) show an endothermic peak observed around 60 ºC that was related to evaporation of residual molecules of water from the polymeric matrix.

It was also observed another exothermic peak around 360-375 ºC that was mainly related to the thermal decomposition or pyrolysis of cellulose molecules due to fragmentation of carbonyl and carboxylic bonds (BARUD et al., 2007). These results were in accordance with the described by Barud et al. (2008), which reported similar DSC patterns of BC samples.

Table 3. XRD parameters and hydrophilic properties of cellulose membranes produced by *K. hansenii* ATCC 23769 after the end of each 56-day pre-cultivation cycles.

Sample	X-Ray Diffraction	Hydrophilic Properties*		
	CrI (%)	WHC	RC (%)	MC (%)
1st cycle	63.5	235.3^{a} ± 22.2	93.9^{a} ± 1.0	99.6^{a} ± 0.1
2nd cycle	73.7	224.4^{a} ± 18.1	91.0^{b} ± 1.2	99.6^{a} ± 0.1
3rd cycle	74.9	190.4^{b} ± 12.7	90.7^{b} ± 1.2	99.5^{a} ± 0.1

* Means followed by equal letters in the same column do not differ significantly from each other based on the Tukey test ($p \leq 0.05$).
CrI = Crystallinity Index; WHC = Water holding capacity;
RC = Reidration capacity; MC = Moisture content.

4.4.3.2.4. TG and DTG analysis

Thermal stability of BC membranes was determined by TG and DTG analysis. Thermal decomposition and the respective DTG curves of the analysed samples (Figures 4(a) and 4(b), respectively) indicated three distinct mass-loss steps. These steps are characteristic of BC degradation process and indicated that produced membranes were stable up to a temperature of 250 ºC. These results are in agreement with Souza et al. (2020) that also investigated the profiles of thermal stability and degradation of pure BC.

The samples of BC produced by the strain *K. hansenii* ATCC 23769 after the end of the 2nd and 3rd cycle exhibited higher parameters of initial (T_{onset}) and final (T_{endset}) thermal decomposition, maximum weight loss rate (T_{max}), and 10% mass loss ($T_{10\%}$) than those produced from the 1st cycle (Table 4), which indicated that there was a need for more energy for it to be degraded. The improvement in thermal stability of BC makes it a very attractive raw material for food packaging applications.

The values of T_{onset} were similar to the reported by Ozen et al. (2021) in which they found values around 290 ºC. The downward peaks in DTG curves were in consistence with the maximum weight loss of TG curves. This weight loss mainly occurred between 300 and 400 ºC (Table 5) indicating the thermal instability of these samples from this temperature, similarly to the reported by Lin et al. (2016) and Molina-Ramírez et al. (2020).

Figure 4. TGA **(a)** and DTG **(b)** curves of bacterial cellulose membranes produced by *K. hansenii* ATCC 23769 at the end of the 56-day pre-cultivation cycles.

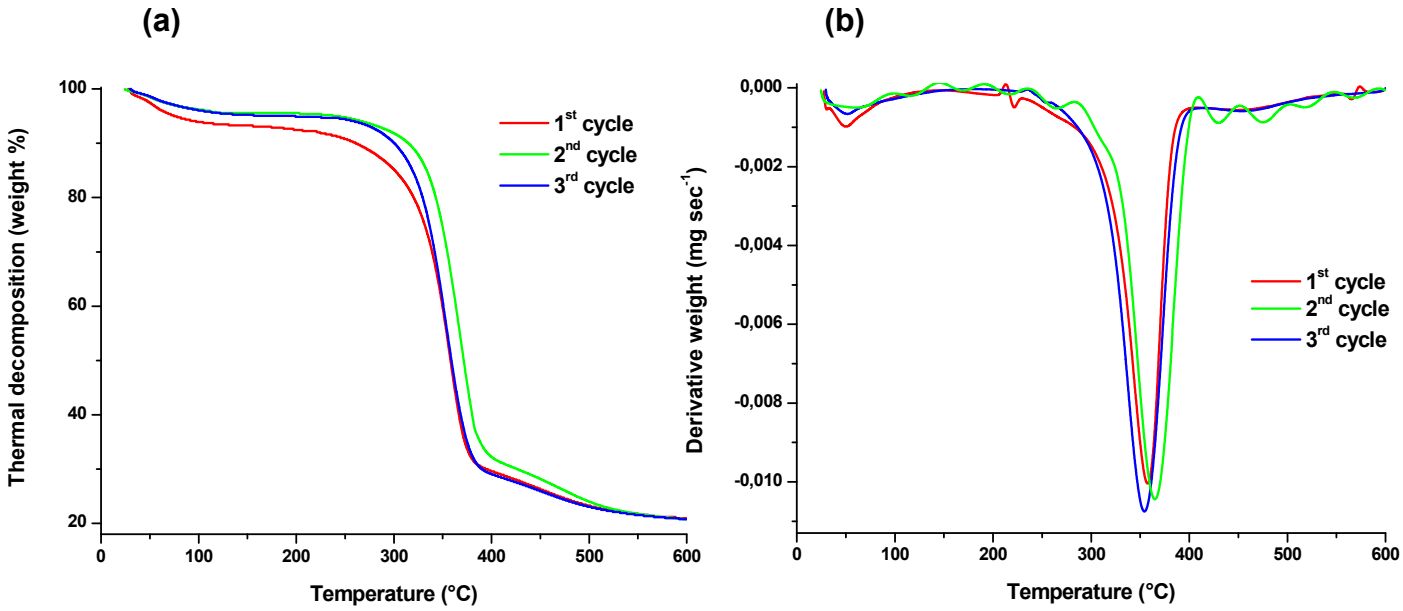


Table 4. TGA parameters of cellulose membranes produced by AAB strain *K. hansenii* ATCC 23769 at the end of the 56-day pre-cultivation cycles.

Sample	T_{onset} [a] (°C)	T_{max} [b] (°C)	T_{endset} [c] (°C)	$T_{10\%}$ [d] (°C)	Weight loss (%)
1st cycle	252.9	357.5	375.3	260.9	79.0
2nd cycle	292.0	364.9	385.9	315.2	79.2
3rd cycle	285.8	354.3	375.2	300.5	79.2

[a] = temperature of initial thermal decomposition;
[b] = temperature of maximum weight loss rate;
[c] = temperature of final thermal decomposition;
[d] = temperature where 10% mass was lost.

Table 5. Weight loss by temperature range of cellulose membranes produced by AAB strain *K. hansenii* ATCC 23769 at the end of the 56-day pre-cultivation cycles.

Temperature range	Weight loss (%)		
	1st cycle	2nd cycle	3rd cycle
30-100°C	5.7	3.5	4.0
100-200°C	1.5	0.6	1.1
200-300°C	7.4	3.8	4.9
300-400°C	55.8	59.8	60.8
400-500°C	6.3	8.3	6.1
500-600°C	2.3	3.2	2.3

4.4.3.2.5. Hydrophilic Properties

In relation to hydrophilic properties (Table 3), all the BC samples had water content superior to 99.5% (w/w). Membranes produced after the end of the 3rd cycle exhibited lower WHC than those produced from the 1st and 2nd cycles.

WHC is a parameter directly linked with porosity and surface area of BC. This indicates that a higher value of WHC was possibly due a larger amount of porous in the polymeric matrix as reported by Ul-Islam, Khan and Park (2012). So, the reduction in WHC by the membranes produced from the 3rd cycle may be related to the higher dry weight and higher crystallinity index of these samples which decreases the amount of porous and empty spaces among BC fibrils. Consequently, there was less space for water accommodation and less quantity of could penetrate and adsorb onto the material, as also observed by Ul-Islam, Khan and Park (2012).

The values of WHC may vary widely from one sample to another. The results obtained in this work were higher than those observed by Wu and Liu (2013) for BC produced using wastewater from rice wine distillery (83-103 times dry weight) but lower than those observed by Mohite and Patil (2014) for BC produced under shaking condition (400 times dry weight). Our results were more similar to those obtained by Bandyopadhyay et al. (2018) for BC produced from waste apple juice (100-250 times dry weight).

WHC is related to never-dried BC while RC represents the degree to which removed water was recovered by the samples. Drying process improves the storage and sell-life of BC, but poor rehydration capacity reduces the utility of dried BC

(HUANG et al., 2010; LIN et al., 2009). In this work, the membranes produced from the 3rd cycle also exhibited lower RC than those produced from the 1st and 2nd cycles. The decrease in hydrophilic properties of the samples produced by the pre-cultivated strain indicates a reduction of hydrophilic interactions, also contributing for them be used to stabilize some types of emulsions in food applications.

4.4.4. Conclusion

A pre-cultivation of AAB for long time can spontaneously select the most productive strains and improve the capacity of BC biosynthesis. The increase in capacity of BC production was influenced by the factors time and number of pre-cultivation cycles before productive fermentation by the strain.

The procedure developed in this work was simple and capable to provide greater amount of BC. In addition, membranes obtained by this method present greater characteristics such as higher crystallinity index and thermal properties, but lower water holding capacity. More studies are recommended to verify the maximum time of pre-cultivation that increases the BC production by AAB strains.

Acknowledgements

We would like to thank “Laboratório de Espectroscopia (ESPEC)” and “Laboratório de Difração de Raios X (LabDRX)” of the State University of Londrina for providing equipments and assistance in the analyses of chemical characterization. The author also thanks “Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)” for providing scholarship during the study period (Process number: 142380/2017-2).

4.4.5. References

AHMED, J.; GULTEKINOGLU, M.; EDIRISINGHE, M.. Bacterial cellulose micro-nano fibres for wound healing applications. **Biotechnology Advances,** v. 41, p. 107549, jul./ago. 2020. https://doi.org/10.1016/j.biotechadv.2020.107549

BANDYOPADHYAY, S.; SAHA, N.; SÁHA, P. Characterization of bacterial cellulose produced using media containing waste apple juice. **Applied Biochemistry and Microbiology,** v. 54, n. 6, p. 649-657, nov. 2018. https://doi.org/10.1134/S0003683818060042

BARUD, H.; RIBEIRO, C.; CRESPI, M.; MARTINES, M.; DEXPERT-GHYS, J.; MARQUES, R.; MESSADDEQ, Y.; RIBEIRO, S. Thermal characterization of bacterial cellulose–phosphate composite membranes. **Journal of Thermal Analysis and Calorimetry,** v. 87, n. 3, p. 815-818, mar. 2007. https://doi.org/10.1007/s10973-006-8170-5

BARUD, H. S.; ARAÚJO JÚNIOR, A. M.; SANTOS, D. B.; ASSUNÇÃO, R. M. N.; MEIRELES, C. S.; CERQUEIRA, D. A.; RODRIGUES FILHO, G.; RIBEIRO, C. A.; MESSADDEQ Y.; RIBEIRO, S. J. L. Thermal behavior of cellulose acetate produced from homogeneous acetylation of bacterial cellulose. **Thermochimica Acta,** v. 471, n. 1-2, p. 61-69, maio 2008. https://doi.org/10.1016/j.tca.2008.02.009

CACICEDO, M. L.; CASTRO, M. C.; SERVETAS, I.; BOSNEA, L.; BOURA, K.; TSAFRAKIDOU, P.; DIMA, A.; TERPOU, A.; KOUTINAS, A.; CASTRO, G. R. Progress in bacterial cellulose matrices for biotechnological applications. **Bioresource Technology,** p. 172-180, v. 213, ago. 2016. https://doi.org/10.1016/j.biortech.2016.02.071

CAMPANO, C.; BALEA, A.; BLANCO, A.; NEGRO, C. Enhancement of the fermentation process and properties of bacterial cellulose: A review. **Cellulose,** v. 23, n. 1, p. 57-91, fev. 2016. https://doi.org/10.1007/s10570-015-0802-0

CHANDRASEKARAN, P. T.; BARI, N. K.; SINHA, S. Enhanced bacterial cellulose production from *Gluconobacter xylinus* using super optimal broth. **Cellulose,** v. 24, n. 10, p. 4367-4381, out. 2017. https://doi.org/10.1007/s10570-017-1419-2

CHEN, G.; WU, G.; CHEN, L.; WANG, W.; HONG, F. F.; JÖNSSON, L. J. Comparison of productivity and quality of bacterial nanocellulose synthesized using culture media based on seven sugars from biomass. **Microbial Biotechnology,** v. 12, n. 4, p. 677-687, jul. 2019. https://doi.org/10.1111/1751-7915.13401

FAN, X.; GAO, Y.; HE, W.; HU, H.; TIAN, M.; WANG, K.; PAN, S. Production of nano bacterial cellulose from beverage industrial waste of citrus peel and pomace using *Komagataeibacter xylinus*. **Carbohydrate Polymers,** v. 151, p. 1068-1072, out. 2016. https://doi.org/10.1016/j.carbpol.2016.06.062

FENG, X.; ULLAH, N.; WANG, X.; SUN, X.; LI, C.; BAI, Y.; CHEN, L.; LI, Z. Characterization of bacterial cellulose by *Gluconacetobacter hansenii* CGMCC

3917. **Journal of Food Science,** v. 80, n. 10, p. E2217-E2227, out. 2015. https://doi.org/10.1111/1750-3841.13010

GOMES, R. J.; BORGES, M. F.; ROSA, M. F.; CASTRO-GÓMEZ, R. J. H.; SPINOSA, W. A. Acetic acid bacteria in the food industry: Systematics, characteristics and applications. **Food Technology and Biotechnology,** v. 56, n. 2, p. 139-151, abr./jun. 2018. https://doi.org/10.17113/ftb.56.02.18.5593

HUANG, H.-C.; CHEN, L.-C.; LIN, S.-B.; HSU, C.-P.; CHEN, H.-H. In situ modification of bacterial cellulose network structure by adding interfering substances during fermentation. **Bioresource Technology,** v. 101, n. 15, p. 6084-6091, ago. 2010. https://doi.org/10.1016/j.biortech.2010.03.031

HUNGUND, B. S.; GUPTA, S. G. Strain improvement of *Gluconacetobacter xylinus* NCIM 2526 for bacterial cellulose production. **African Journal of Biotechnology,** v. 9, n. 32, p. 5170-5172, ago. 2010.

KAČURÁKOVÁ, M.; SMITH, A. C.; GIDLEY, M. J.; WILSON, R. H. Molecular interactions in bacterial cellulose composites studied by 1D FT-IR and dynamic 2D FT-IR spectroscopy. **Carbohydrate Research,** v. 337, n. 12, p. 1145-1153, 2002.

KONDO, T. The assigment of IR absorption bands due to free hidroxyl groups in cellulose. **Cellulose,** v. 4, p. 281-292, dez. 1997.

KRYSTYNOWICZ, A.; CZAJA, W.; WIKTOROWSKA-JEZIERSKA, A.; GONÇALVES-MIŚKIEWICZ, M.; TURKIEWICZ, M.; BIELECKI, S. Factors affecting the yield and properties of bacterial cellulose. **Journal of Industrial Microbiology and Biotechnology,** v. 29, n. 4, p. 189-195, out. 2002. https://doi.org/10.1038/sj.jim.7000303

LI, X.; LI, J.; GONG, J.; KUANG, Y.; MO, L.; SONG, T. Cellulose nanocrystals (CNCs) with different crystalline allomorph for oil in water Pickering emulsions. **Carbohydrate Polymers,** v. 183, p. 303-310, mar. 2018. https://doi.org/10.1016/j.carbpol.2017.12.085

LIN, S. B.; HSU, C. P.; CHEN, L. C.; CHEN, H. H. Adding enzymatically modified gelatin to enhance the rehydration abilities and mechanical properties of bacterial cellulose. **Food Hydrocolloids,** v. 23, n. 8, p. 2195-2203, dez. 2009. https://doi.org/10.1016/j.foodhyd.2009.05.011

LIN, S. P.; LIU, C. T.; HSU, K. D.; HUNG, Y. T.; SHIH, T. Y.; CHENG, K. C. Production of bacterial cellulose with various additives in a PCS rotating disk bioreactor and its material property analysis. **Cellulose,** v. 23, n. 1, p. 367-377, fev. 2016. https://doi.org/10.1007/s10570-015-0855-0

LIU, M.; LIU, L.; JIA, S.; LI, S.; ZOU, Y.; ZHONG, C. Complete genome analysis of *Gluconacetobacter xylinus* CGMCC 2955 for elucidating bacterial cellulose biosynthesis and metabolic regulation. **Scientific Reports,** v. 8, n. 1, p. 1-10, 2018. https://doi.org/10.1038/s41598-018-24559-w

MARTÍNEZ-SANZ, M.; OLSSON, R. T.; LOPEZ-RUBIO, A.; LAGARON, J. M. Development of electrospun EVOH fibres reinforced with bacterial cellulose nanowhiskers. Part I: Characterization and method optimization. **Cellulose,** v. 18, n. 2, p. 335-347, abr. 2011. https://doi.org/10.1007/s10570-010-9471-1

MOHAMMADKAZEMI, F.; AZIN, M.; ASHORI, A. Production of bacterial cellulose using different carbon sources and culture media. **Carbohydrate Polymers,** v. 117, p. 518-523, mar. 2015. https://doi.org/10.1016/j.carbpol.2014.10.008

MOHITE, B. V.; PATIL, S. V. Physical, structural, mechanical and thermal characterization of bacterial cellulose by *G. hansenii* NCIM 2529. **Carbohydrate Polymers,** v. 106, p. 132-141, jun. 2014. https://doi.org/10.1016/j.carbpol.2014.02.012

MOLINA-RAMÍREZ, C.; CAÑAS-GUTIÉRREZ, A.; CASTRO, C.; ZULUAGA, R.; GAÑÁN, P. Effect of production process scale-up on the characteristics and properties of bacterial nanocellulose obtained from overripe Banana culture medium. **Carbohydrate Polymers,** v. 240, p. 116341, jul. 2020. https://doi.org/10.1016/j.carbpol.2020.116341

NÚÑEZ, D.; CÁCERES, R.; IDE, W.; VARAPRASAD, K.; OYARZÚN, P. An ecofriendly nanocomposite of bacterial cellulose and hydroxyapatite efficiently removes lead from water. **International Journal of Biological Macromolecules,** v. 165, p. 2711-2720, dez. 2020. https://doi.org/10.1016/j.ijbiomac.2020.10.055

OZEN, E.; YILDIRIM, N.; DALKILIC, B.; ERGUN, M. E. Effects of microcrystalline cellulose on some performance properties of chitosan aerogels. **Maderas. Ciencia y tecnología,** v. 23, fev. 2021. http://doi.org/10.4067/s0718-221x2021000100426

POGORELOVA, N.; ROGACHEV, E.; DIGEL, I.; CHERNIGOVA, S.; NARDIN, D. Bacterial cellulose nanocomposites: Morphology and mechanical properties. **Materials,** v. 13, n. 12, p. 2849, 2020. https://doi.org/10.3390/ma13122849

QIU, X.; ZHANG, Y.; HONG, H. Classification of acetic acid bacteria and their acid resistant mechanism. **AMB Express,** v. 11, n. 1, p. 1-15, fev. 2021. https://doi.org/10.1186/s13568-021-01189-6

SENGUN, I. Y.; KARABIYIKLI, S. Importance of acetic acid bacteria in food industry. **Food Control,** v. 22, n. 5, p. 647-656, maio 2011. https://doi.org/10.1016/j.foodcont.2010.11.008

SARRAFZADEH, M. H. Nutritional requirements of *Bacillus thuringiensis* during different phases of growth, sporulation and germination evaluated by Plackett-Burman method. **Iranian Journal of Chemistry and Chemical Engineering,** v. 31, n. 4, p. 131-136, nov./dez. 2012.

SEGAL, L. G. J. M. A.; CREELY, J. J.; MARTIN JR, A. E.; CONRAD, C. M. An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer. **Textile Research Journal,** v. 29, n. 10, p. 786-794, out. 1959. https://doi.org/10.1177/004051755902901003

SEMJONOVS, P.; RUKLISHA, M.; PAEGLE, L.; SAKA, M.; TREIMANE, R.; SKUTE, M.; ROZENBERGA, L.; VIKELE, L.; SABOVICS, M.; CLEENWERCK, I. Cellulose synthesis by *Komagataeibacter rhaeticus* strain P 1463 isolated from Kombucha. **Applied Microbiology and Biotechnology,** v. 101, n. 3, p. 1003-1012, fev. 2017. https://doi.org/10.1007/s00253-016-7761-8

SHARAFI, S. M.; RASOOLI, I.; BEHESHTI-MAAL, K. Isolation, characterization and optimization of indigenous acetic acid bacteria and evaluation of their preservation methods. **Iranian Journal of Microbiology,** v. 2, n. 1, p. 38-45, mar. 2010.

SHIGEMATSU, T.; TAKAMINE, K.; KITAZATO, M.; MORITA, T.; NARITOMI, T.; MORIMURA, S., KIDA, K. Cellulose production from glucose using a glucose dehydrogenase gene (gdh)-deficient mutant of *Gluconacetobacter xylinus* and its use for bioconversion of sweet potato pulp. **Journal of Bioscience and Bioengineering,** v. 99, n. 4, p. 415-422, abr. 2005. https://doi.org/10.1263/jbb.99.415

SOMMER, A.; STAROSZCZYK, H.; SINKIEWICZ, I.; BRUŹDZIAK, P. Preparation and characterization of films based on disintegrated bacterial cellulose and montmorillonite. **Journal of Polymers and the Environment,** v. 29, n. 5, p. 1526-1541, maio 2021. https://doi.org/10.1007/s10924-020-01968-5

SOUZA, E. F.; FURTADO, M. R.; CARVALHO, C. W.; FREITAS-SILVA, O.; GOTTSCHALK, L. M. Production and characterization of *Gluconacetobacter xylinus* bacterial cellulose using cashew apple juice and soybean molasses. **International Journal of Biological Macromolecules,** v. 146, p. 285-289, mar. 2020. https://doi.org/10.1016/j.ijbiomac.2019.12.180

STANISŁAWSKA, A.; STAROSZCZYK, H.; SZKODO, M. The effect of dehydration/rehydration of bacterial nanocellulose on its tensile strength and

physicochemical properties. **Carbohydrate Polymers,** v. 236, p. 116023, mar. 2020. https://doi.org/10.1016/j.carbpol.2020.116023

STASIAK, L.; BŁAŻEJAK, S. Acetic acid bacteria – Perspectives of application in biotechnology – A review. **Polish Journal of Food and Nutrition Sciences,** v. 59, n. 1, p. 17-23, 2009.

STASIAK-RÓŻAŃSKA, L.; BERTHOLD-PLUTA, A.; DIKSHIT, P. K. Valorization of waste glycerol to dihydroxyacetone with biocatalysts obtained from *Gluconobacter oxydans*. **Applied Sciences,** v. 8, n. 12, p. 2517, 2018. https://doi.org/10.3390/app8122517

SUGIYAMA, J.; PERSSON, J.; CHANZY, H. Combined infrared and electron diffraction study of the polymorphism of native celluloses. **Macromolecules,** v. 24, n. 9, p. 2461-2466, abr. 1991.

TEIXEIRA, S. R. Z.; REIS, E. M. D.; APATI, G. P.; MEIER, M. M.; NOGUEIRA, A. L.; GARCIA, M. C. F.; SCHNEIDER, A. L. S.; PEZZIN, A. P. T.; PORTO, L. M. Biosynthesis and Functionalization of Bacterial Cellulose Membranes with Cerium Nitrate and Silver Nanoparticles. **Materials Research,** v. 22, p. e20190054, 2019. https://doi.org/10.1590/1980-5373-MR-2019-0054

UL-ISLAM, M.; KHAN, T.; PARK, J. K. Water holding and release properties of bacterial cellulose obtained by in situ and ex situ modification. **Carbohydrate Polymers,** v. 88, n. 2, p. 596-603, abr. 2012. https://doi.org/10.1016/j.carbpol.2012.01.006

ULLAH, H.; WAHID, F.; SANTOS, H. A.; KHAN, T. Advances in biomedical and pharmaceutical applications of functional bacterial cellulose-based nanocomposites. **Carbohydrate Polymers,** v. 150, p. 330-352, out. 2016. https://doi.org/10.1016/j.carbpol.2016.05.029

VALLA, S.; KJOSBAKKEN, J. Cellulose-negative mutants of *Acetobacter xylinum*. **Microbiology,** v. 128, n. 7, p. 1401-1408, jul. 1982. https://doi.org/10.1099/00221287-128-7-1401

VAZQUEZ, A.; FORESTI, M. L.; CERRUTTI, P.; GALVAGNO, M. Bacterial cellulose from simple and low cost production media by *Gluconacetobacter xylinus*. **Journal of Polymers and the Environment,** v. 21, n. 2, p. 545-554, jun. 2013. https://doi.org/10.1007/s10924-012-0541-3

WANG, J.; TAVAKOLI, J.; TANG, Y. Bacterial cellulose production, properties and applications with different culture methods–A review. **Carbohydrate Polymers,** v. 219, p. 63-76, set. 2019. https://doi.org/10.1016/j.carbpol.2019.05.008

WU, R. Q.; LI, Z. X.; YANG, J. P.; XING, X. H.; SHAO, D. Y.; XING, K. L. Mutagenesis induced by high hydrostatic pressure treatment: a useful method to improve the bacterial cellulose yield of a *Gluconoacetobacter xylinus* strain. **Cellulose,** v. 17, n. 2, p. 399-405, abr. 2010. https://doi.org/10.1007/s10570-009-9388-8

XU, S.; WANG, X.; DU, G.; ZHOU, J.; CHEN, J. Enhanced production of L-sorbose from D-sorbitol by improving the mRNA abundance of sorbitol dehydrogenase in Gluconobacter oxydans WSH-003. **Microbial Cell Factories,** v. 13, n. 1, p. 1-7, 2014. https://doi.org/10.1186/s12934-014-0146-8

5. CONCLUSÃO GERAL

Bactérias acéticas de espécies diferentes pertencentes ao mesmo gênero apresentaram necessidades nutricionais distintas além de diferentes capacidades de biossíntese de biocelulose, dependendo da composição do meio de cultura utilizado.

Em nível qualitativo, o estudo indicou que os minerais ferro, zinco e manganês, e as vitaminas ácido pantotênico e ácido nicotínico apresentaram um efeito positivo significativo sobre a produção de biocelulose e foram requeridos pelas cepas. Por outro lado, as vitaminas tiamina e riboflavina apresentaram um efeito negativo significativo e foram rejeitados pelas cepas.

Em nível quantitativo, o estudo indicou que a espécie *K. hansenii* ATCC 23769 requereu em torno de 20,0 mg L^{-1} de ferro, 20,0 mg L^{-1} de ácido pantotênico, 7,0 mg L^{-1} de ácido nicotínico e 5,0 mg L^{-1} de zinco, enquanto que a espécie *K. intermedius* requereu em torno de 15,0 mg L^{-1} de ferro, 15,0 mg L^{-1} de manganês, 6,0 mg L^{-1} de ácido pantotênico e 6,0 mg L^{-1} de ácido nicotínico.

Para a cepa *K. hansenii* ATCC 23769, que apresentou baixo rendimento em meio padrão HS, o meio otimizado suplementado com micronutrientes aumentou a produção de biocelulose e melhorou as características térmicas e cristalinas das membranas em comparação com a produção em meio não otimizado. Para a cepa *K. intermedius* V-05, que apresenta alto rendimento em meio padrão HS, não houve aumento no rendimento mas a produção no meio otimizado suplementado com micronutrientes melhorou as características térmicas e hidrofílicas das membranas.

Assim como os minerais e as vitaminas, os diferentes aminoácidos tiveram efeitos distintos na capacidade de biossíntese de celulose por BAA impactando nas características das membranas obtidas.

Em nível qualitativo, o estudo indicou que ácido aspártico, fenilalanina e serina apresentaram um efeito positivo significativo sobre a produção de biocelulose e foram requeridos pela cepa *K. intermedius* V-05. Por outro lado, outros aminoácidos principalmente aqueles apolares, contendo enxofre ou carga positiva tiveram um efeito negativo e foram rejeitados pela cepa.

Em nível quantitativo, o estudo indicou que a cepa *K. intermedius* V-05 requereu em torno de 1,5 g L^{-1} de ácido aspártico, 1,5 g L^{-1} de fenilalanina e 3,0 g L^{-1} de serina para se obter um bom rendimento de biocelulose. Embora o rendimento

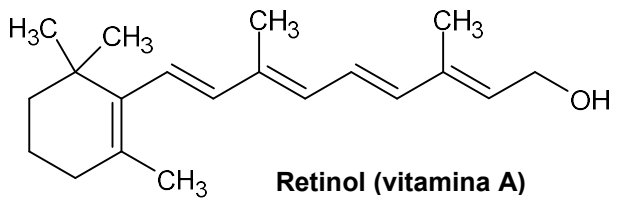
não tenha sido maior do que o alcançado em meio padrão HS, seu uso forneceu membranas com melhores características térmicas e hidrofílicas.

Através do planejamento fatorial de Plackett e Burman seguido da metodologia de superfície de resposta foi possível elaborar meios sintéticos de crescimento capazes de fornecer micronutrientes e aminoácidos essenciais necessários à biossíntese de celulose.

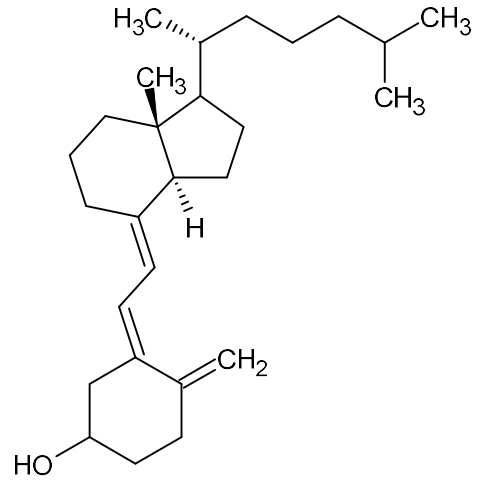
Um tempo de pré-cultivo de pelo menos 56 dias (ou oito semanas) antes da fermentação para produção de biocelulose por linhagem de baixa produtividade, naturalmente selecionou células com maior capacidade de produção e aumentou o rendimento das fermentações realizadas após o período de pré-cultivo. Além disso, as membranas obtidas por este método apresentaram melhores características, tais como índice de cristalinidade e propriedades térmicas comparadas com aquelas obtidas pelas cepas não pré-cultivadas.

APÊNDICES

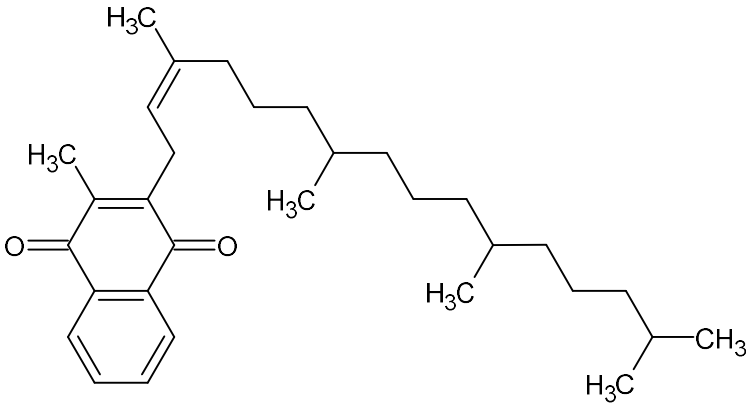
Figura A1. Estrutura química das vitaminas lipossolúveis.



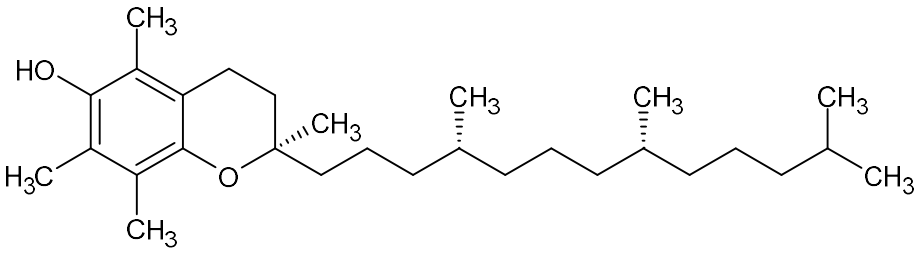
Retinol (vitamina A)



Colecalciferol (vitamina D3)



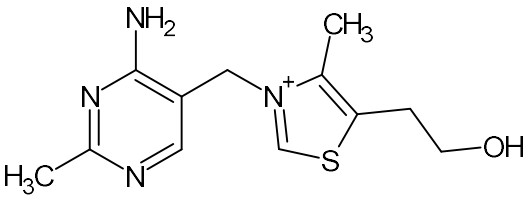
Filoquinona (vitamina K1)



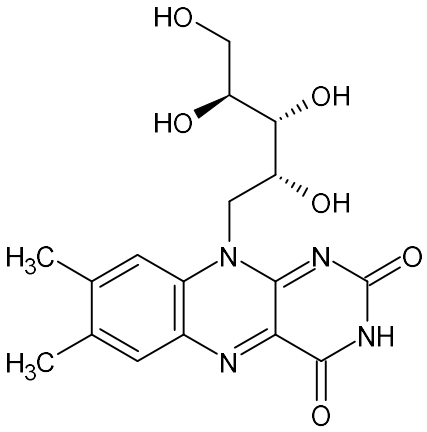
Tocoferol (vitamina E)

Fonte: Fennema; Damodaran; Parkin (2017). Adaptado.

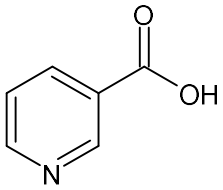
Figura A2. Estrutura química das vitaminas hidrossolúveis.



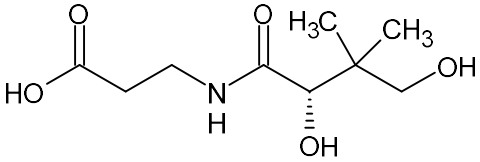
Tiamina (vitamina B1)



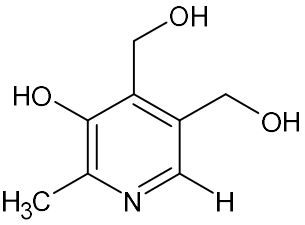
Riboflavina (vitamina B2)



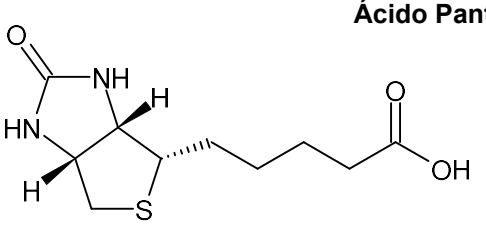
Ácido Nicotínico (vitamina B3)



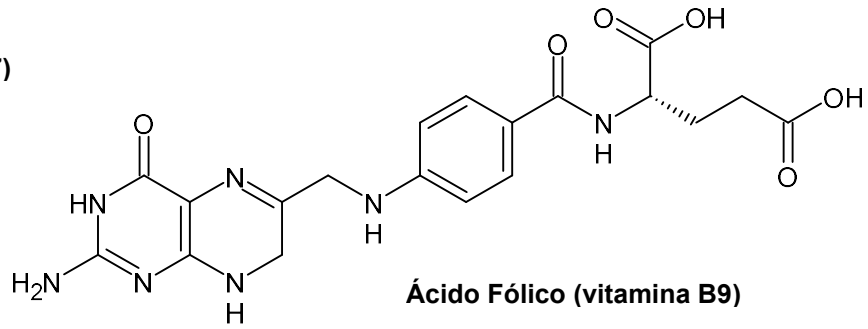
Ácido Pantotênico (vitamina B5)



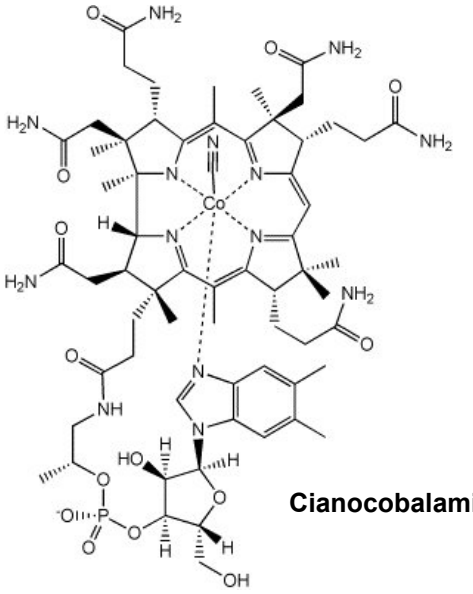
Piridoxina (vitamina B6)



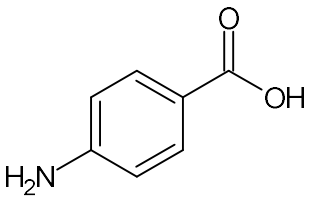
Biotina (vitamina B7)



Ácido Fólico (vitamina B9)

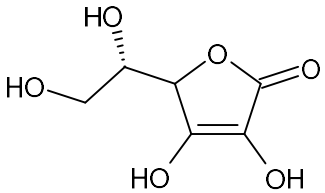


Cianocobalamina (vitamina B12)



Ácido *p*-Aminobenzóico (PABA)

Ácido Ascórbico (vitamina C)



Fonte: Fennema; Damodaran; Parkin (2017). Adaptado.

Figure A3. Plot of predicted versus observed values of BC production (g L^{-1}) obtained in central composite design with selected minerals and vitamins by AAB strain *K. hansenii* ATCC 23769.

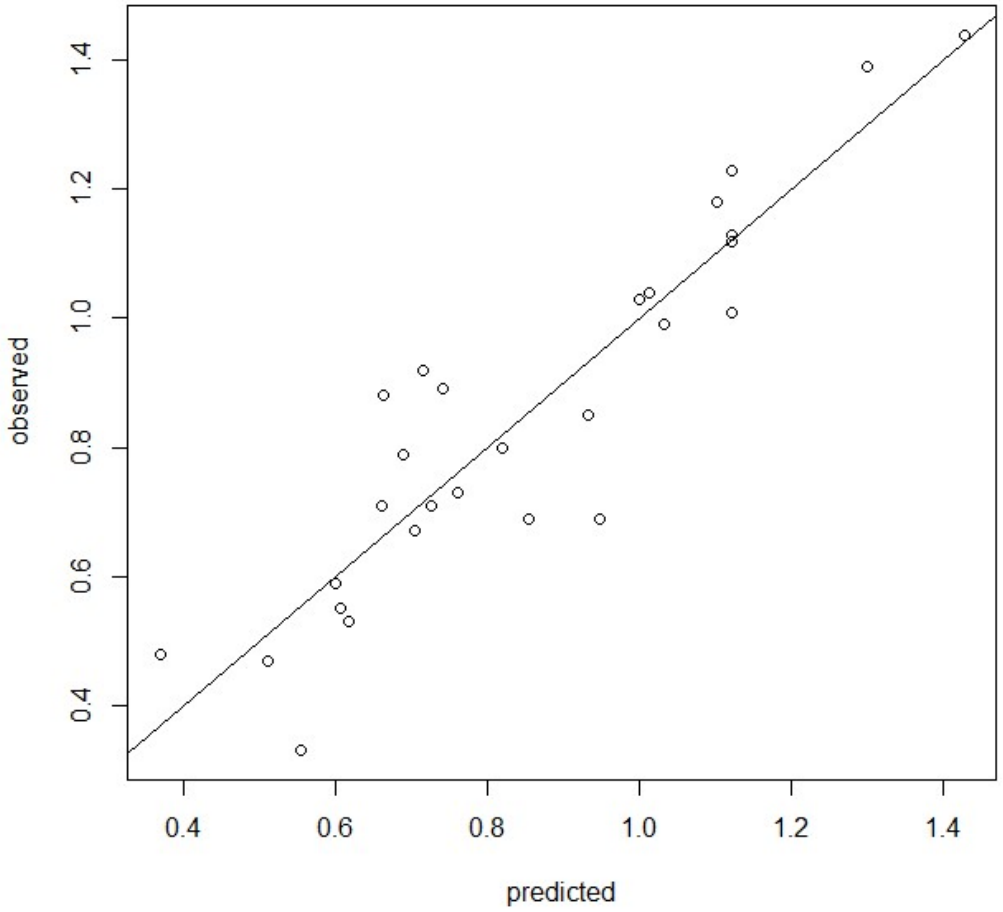


Figure A4. Plot of predicted versus observed values of BC production (g L^{-1}) obtained in central composite design with selected minerals and vitamins by AAB strain *K. intermedius* V-05.

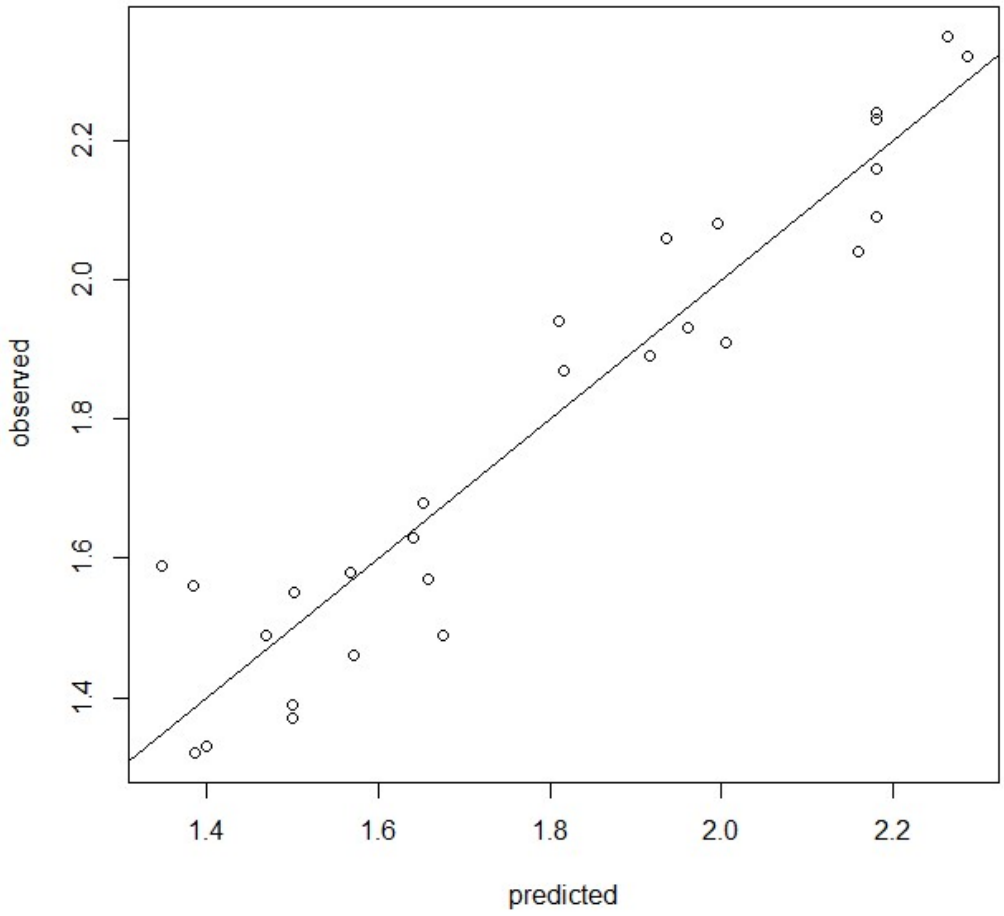


Figure A5. Plot of predicted versus observed values of BC production (g L^{-1}) obtained in central composite design with selected amimo acids by AAB strain *K. intermedius* V-05.

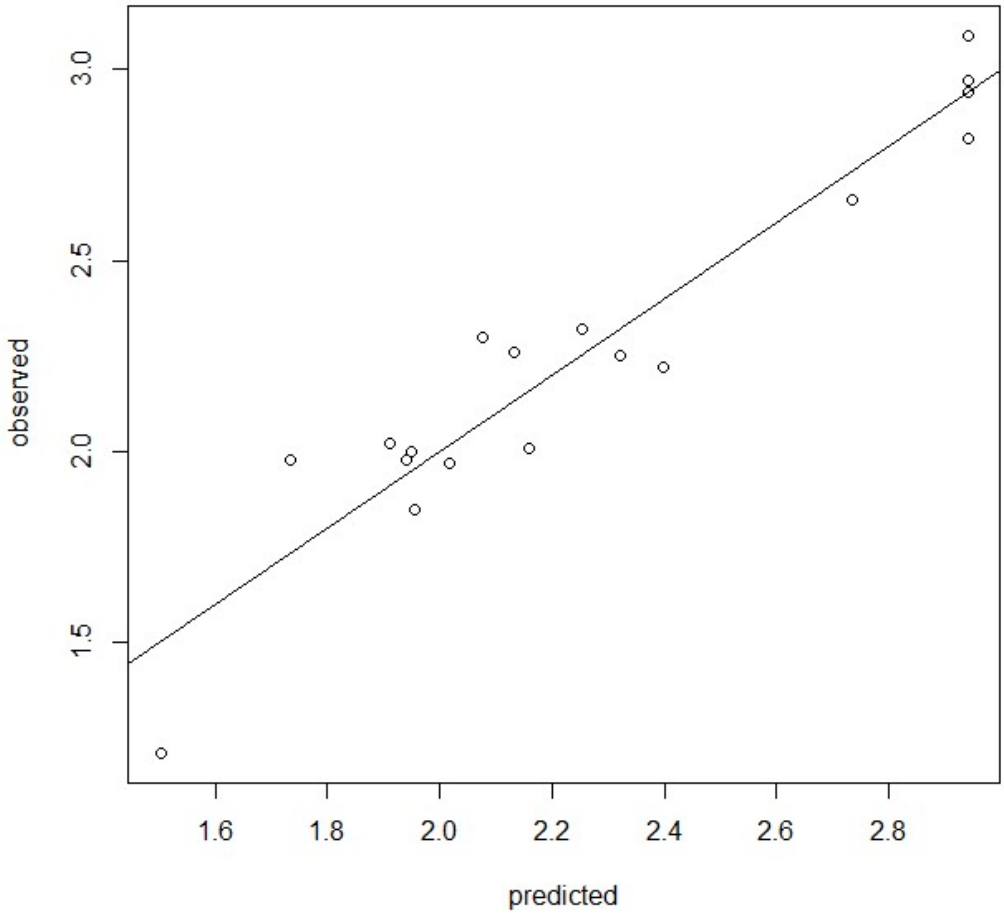


Tabela A1. Nomenclatura e estrutura química dos principais aminoácidos.

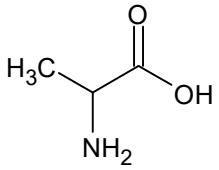
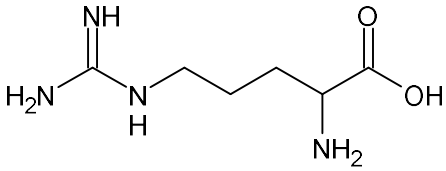
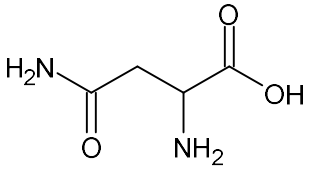
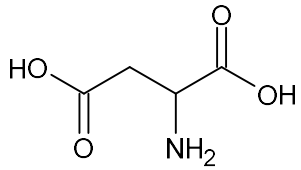
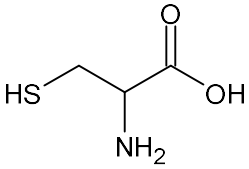
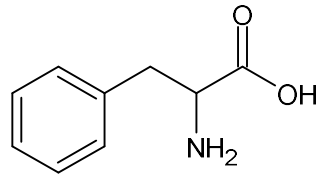
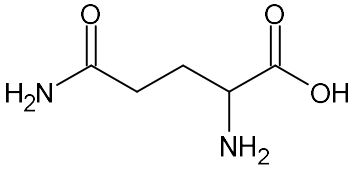
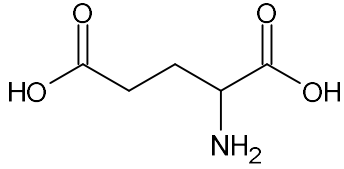
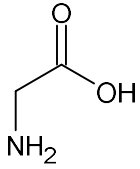
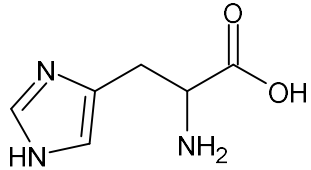
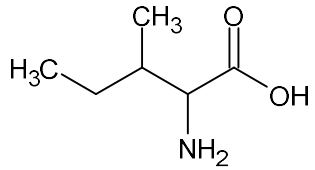
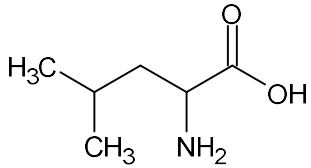
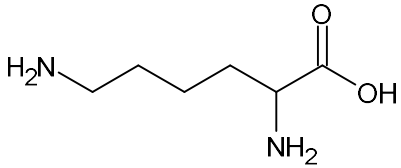
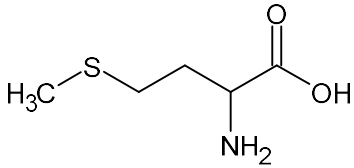
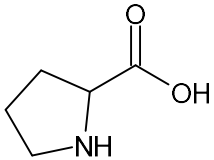
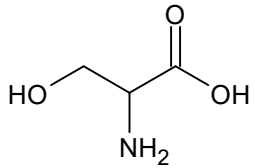
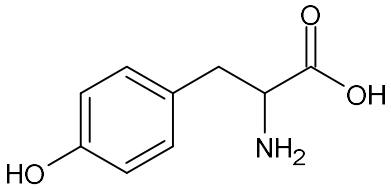
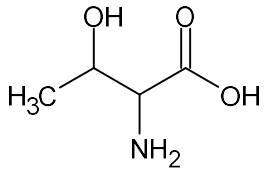
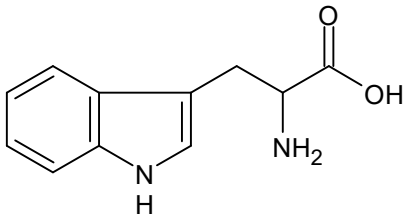
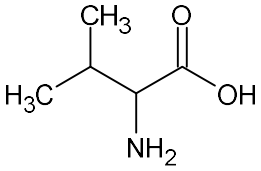
Aminoácido	Estrutura	Aminoácido	Estrutura
Alanina		**Arginina**	
Asparagina		**Ácido aspártico**	
Cisteína		**Fenilalanina**	
Glutamina		**Ácido glutâmico**	
Glicina		**Histidina**	

Tabela A1. Nomenclatura e estrutura química dos principais aminoácidos (continuação).

Aminoácido	Estrutura	Aminoácido	Estrutura
Isoleucina		**Leucina**	
Lisina		**Metionina**	
Prolina		**Serina**	
Tirosina		**Treonina**	
Triptofano		**Valina**	

Fonte: Fennema; Damodaran; Parkin (2017). Adaptado.

Table A2. Matrix of Plackett & Burman design used to study the effects of micronutrients on bacterial cellulose production.

Run	Independent variables coded																		
	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}	D_1	D_2	D_3	D_4
1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	1
2	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1
3	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1
4	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1
5	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1
6	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1
7	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1
8	1	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1
9	-1	1	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1
10	1	-1	1	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1
11	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1
12	1	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1
13	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1
14	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	1	1	-1	-1
15	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	1	1	-1
16	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	1	1
17	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	1
18	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	1	-1
19	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	1
20	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1

Legend: X_1 – boron; X_2 – iron; X_3 – manganese; X_4 – molybdenum; X_5 – zinc; X_6 – folic acid; X_7 – pantothenate; X_8 – *p*-aminobenzoic acid; X_9 – biotin; X_{10} – cyanocobalamin; X_{11} – inositol; X_{12} – niacin; X_{13} – pyridoxine; X_{14} – riboflavin; X_{15} – thiamine; D_X – dummy variable.

Table A3. Statistical analysis of effects generated by Plackett & Burman design for bacterial cellulose produced by *K. hansenii* ATCC 23769 and *K. intermedius* V-05 in media supplemented with several minerals and vitamins.

	***K. hansenii* ATCC 23769**			***K. intermedius* V-05**		
	Estimated effect	**t value**	***p*-value**	**Estimated effect**	**t value**	***p*-value**
X_1	0.019	0.879	0.40487	$4.243e^{-17}$	0.000	1.00000
X_2	0.073	3.378	0.00966	0.121	4.002	0.00394
X_3	-0.008	-0.370	0.72081	0.067	2.216	0.05756
X_4	0.006	0.278	0.78830	0.023	0.761	0.46870
X_5	0.075	3.471	0.00843	0.045	1.488	0.17502
X_6	$-4.883e^{-17}$	0.000	1.00000	-0.048	-1.587	0.15108
X_7	0.052	2.407	0.04274	0.221	7.309	$8.32e^{-05}$
X_8	0.016	0.740	0.48018	-0.037	-1.224	0.25591
X_9	-0.027	-1.250	0.24678	-0.024	-0.794	0.45027
X_{10}	0.013	0.602	0.56407	0.007	0.231	0.82274
X_{11}	-0.006	-0.278	0.78830	0.019	0.628	0.54729
X_{12}	0.063	2.916	0.01942	0.048	1.587	0.15108
X_{13}	-0.012	-0.555	0.59383	0.008	0.265	0.79803
X_{14}	-0.051	-2.360	0.04594	-0.048	-1.587	0.15108
X_{15}	-0.060	-2.777	0.02404	-0.052	-1.720	0.12381
R^2	0.8728	-	-	0.9186	-	-
Adj. R^2	0.6342	-	-	0.7660	-	-

Legend: X_1 – boron; X_2 – iron; X_3 – manganese; X_4 – molybdenum; X_5 – zinc; X_6 – folic acid; X_7 – pantothenate; X_8 – *p*-aminobenzoic acid; X_9 – biotin; X_{10} – cyanocobalamin; X_{11} – inositol; X_{12} – niacin; X_{13} – pyridoxine; X_{14} – riboflavin; X_{15} – thiamine.

Table A4. Statistical analysis of central composite design for bacterial cellulose production using selected minerals and vitamins by *K. hansenii* ATCC 23769.

Analysis of Effects Table			
	Estimated effect	**t value**	***p*-value**
X_1	0.107917	3.2524	0.006299
X_1^2	-0.076563	-2.3074	0.038129
X_2	-0.057917	-1.7455	0.104473
X_2^2	-0.072813	-2.1944	0.046976
X_3	0.121250	3.6542	0.002914
X_3^2	-0.127812	-3.8520	0.002000
X_4	0.017083	0.5149	0.615295
X_4^2	-0.039062	-1.1773	0.260195
X_1*X_2	-0.060625	-1.4918	0.159607
X_1*X_3	0.175625	4.3217	0.000829
X_1*X_4	0.025625	0.6306	0.539250
X_2*X_3	-0.034375	-0.8459	0.412917
X_2*X_4	-0.009375	-0.2307	0.821142
X_3*X_4	0.011875	0.2922	0.774734
R^2	0.8380		
Adjusted R^2	0.6635		

Analysis of Variance Table				
	SS*	**MS****	**F value**	***p*-value**
Linear (X_1, X_2, X_3, X_4)	0.71985	0.179962	6.8108	0.003488
Quadratic (X_1, X_2, X_3, X_4)	0.47161	0.117901	4.4621	0.017311
Interaction (X_1, X_2, X_3, X_4)	0.58539	0.097565	3.6924	0.022980
Residues	0.34350	0.026423		
Lack of adjustment	0.31922	0.031922	3.9451	0.142867
Pure error	0.02427	0.008092		

Legend: **X_1** – iron; **X_2** – zinc; **X_3** – pantothenate; **X_4** – niacin.
***** Sum of squares. ****** Mean square.

Table A5. Statistical analysis of central composite design for bacterial cellulose production using selected minerals and vitamins by *K. intermedius* V-05.

Analysis of Effects Table			
	Estimated effect	**t value**	***p*-value**
X_1	0.137083	4.6021	0.000496
X_1^2	-0.129688	-4.3538	0.000781
X_2	0.193750	6.5045	$1.989e^{-05}$
X_2^2	-0.102188	-3.4306	0.004471
X_3	-0.082083	-2.7557	0.016359
X_3^2	-0.167187	-5.6128	$8.441e^{-05}$
X_4	0.019583	0.6574	0.522364
X_4^2	-0.055938	-1.8779	0.083005
X_1*X_2	0.073125	2.0044	0.066310
X_1*X_3	-0.020625	-0.5654	0.581457
X_1*X_4	-0.006875	-0.1885	0.853432
X_2*X_3	-0.043125	-1.1821	0.258333
X_2*X_4	-0.006875	-0.1885	0.853432
X_3*X_4	0.016875	0.4626	0.651323
R^2	0.9028		
Adjusted R^2	0.7982		

Analysis of Variance Table				
	SS*	**MS****	**F value**	***p*-value**
Linear (X_1, X_2, X_3, X_4)	1.52285	0.38071	17.8787	$3.377e^{-05}$
Quadratic (X_1, X_2, X_3, X_4)	0.92054	0.23013	10.8074	0.000440
Interaction (X_1, X_2, X_3, X_4)	0.12819	0.02136	1.0033	0.463526
Residues	0.27683	0.02129		
Lack of adjustment	0.26223	0.02622	5.3882	0.096220
Pure error	0.01460	0.00487		

Legend: X_1 – iron; X_2 – manganese; X_3 – pantothenate; X_4 – niacin.
* Sum of squares. ** Mean square.

Table A6. Matrix of Plackett & Burman design used to study the effects of micronutrients on bacterial cellulose production.

Run	Independent variables coded																						
	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}	X_{16}	X_{17}	X_{18}	X_{19}	D_1	D_2	D_3	D_4
1	1	-1	-1	-1	-1	1	-1	1	-1	-1	1	1	-1	-1	1	1	-1	1	-1	1	1	1	1
2	1	1	-1	-1	-1	-1	1	-1	1	-1	-1	1	1	-1	-1	1	1	-1	1	-1	1	1	1
3	1	1	1	-1	-1	-1	-1	1	-1	1	-1	-1	1	1	-1	-1	1	1	-1	1	-1	1	1
4	1	1	1	1	-1	-1	-1	-1	1	-1	1	-1	-1	1	1	-1	-1	1	1	-1	1	-1	1
5	1	1	1	1	1	-1	-1	-1	-1	1	-1	1	-1	-1	1	1	-1	-1	1	1	-1	1	-1
6	-1	1	1	1	1	1	-1	-1	-1	-1	1	-1	1	-1	-1	1	1	-1	-1	1	1	-1	1
7	1	-1	1	1	1	1	1	-1	-1	-1	-1	1	-1	1	-1	-1	1	1	-1	-1	1	1	-1
8	-1	1	-1	1	1	1	1	1	-1	-1	-1	-1	1	-1	1	-1	-1	1	1	-1	-1	1	1
9	1	-1	1	-1	1	1	1	1	1	-1	-1	-1	-1	1	-1	1	-1	-1	1	1	-1	-1	1
10	1	1	-1	1	-1	1	1	1	1	1	-1	-1	-1	-1	1	-1	1	-1	-1	1	1	-1	-1
11	-1	1	1	-1	1	-1	1	1	1	1	1	-1	-1	-1	-1	1	-1	1	-1	-1	1	1	-1
12	-1	-1	1	1	-1	1	-1	1	1	1	1	1	-1	-1	-1	-1	1	-1	1	-1	-1	1	1
13	1	-1	-1	1	1	-1	1	-1	1	1	1	1	1	-1	-1	-1	-1	1	-1	1	-1	-1	1
14	1	1	-1	-1	1	1	-1	1	-1	1	1	1	1	1	-1	-1	-1	-1	1	-1	1	-1	-1
15	-1	1	1	-1	-1	1	1	-1	1	-1	1	1	1	1	1	-1	-1	-1	-1	1	-1	1	-1
16	-1	-1	1	1	-1	-1	1	1	-1	1	-1	1	1	1	1	1	-1	-1	-1	-1	1	-1	1
17	1	-1	-1	1	1	-1	-1	1	1	-1	1	-1	1	1	1	1	1	-1	-1	-1	-1	1	-1
18	-1	1	-1	-1	1	1	-1	-1	1	1	-1	1	-1	1	1	1	1	1	-1	-1	-1	-1	1
19	1	-1	1	-1	-1	1	1	-1	-1	1	1	-1	1	-1	1	1	1	1	1	-1	-1	-1	-1
20	-1	1	-1	1	-1	-1	1	1	-1	-1	1	1	-1	1	-1	1	1	1	1	1	-1	-1	-1
21	-1	-1	1	-1	1	-1	-1	1	1	-1	-1	1	1	-1	1	-1	1	1	1	1	1	-1	-1
22	-1	-1	-1	1	-1	1	-1	-1	1	1	-1	-1	1	1	-1	1	-1	1	1	1	1	1	-1
23	-1	-1	-1	-1	1	-1	1	-1	-1	1	1	-1	-1	1	1	-1	1	-1	1	1	1	1	1
24	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1

Legend: X_1 – alanine; X_2 – arginine; X_3 – asparagine; X_4 – aspartic acid; X_5 – cysteine; X_6 – glycine; X_7 – glutamine; X_8 – glutamic acid; X_9 – histidine; X_{10} – leucine; X_{11} – isoleucine; X_{12} – methionine; X_{13} – proline; X_{14} – phenylalanine; X_{15} – lysine; X_{16} – serine; X_{17} – threonine; X_{18} – valine; X_{19} – tryptophan; ; D_X – dummy variable.

Table A7. Statistical analysis of effects generated by analysis of Plackett & Burman design for bacterial cellulose production by *K. intermedius* V-05 in media supplemented with several amino acids.

	Estimated effect	t value	*p*-value
X_1	-0.04583	-2.820	0.022502
X_2	-0.05167	-3.179	0.013024
X_3	$5.246e^{-17}$	0.000	1.000000
X_4	0.05833	3.589	0.007097
X_5	-0.09167	-5.640	0.000487
X_6	0.00750	0.461	0.656788
X_7	0.02167	1.333	0.219251
X_8	-0.02333	-1.436	0.189063
X_9	-0.03333	-2.051	0.074424
X_{10}	-0.04500	-2.768	0.024354
X_{11}	0.00417	0.256	0.804156
X_{12}	-0.10920	-6.716	0.000150
X_{13}	0.01167	0.718	0.493332
X_{14}	0.03500	2.153	0.063452
X_{15}	-0.00667	-0.410	0.692463
X_{16}	0.02667	1.641	0.139513
X_{17}	0.01167	0.718	0.493332
X_{18}	-0.01000	-0.615	0.555503
X_{19}	-0.05083	-3.127	0.014071
R^2	0.9468	-	-
Adjusted R^2	0.8206	-	-

Legend: X_1 – alanine; X_2 – arginine; X_3 – asparagine; X_4 – aspartic acid; X_5 – cysteine; X_6 – glycine; X_7 – glutamine; X_8 – glutamic acid; X_9 – histidine; X_{10} – leucine; X_{11} – isoleucine; X_{12} – methionine; X_{13} – proline; X_{14} – phenylalanine; X_{15} – lysine; X_{16} – serine; X_{17} – threonine; X_{18} – valine; X_{19} – tryptophan.

Table A8. Statistical analysis of central composite design for bacterial cellulose production using selected amino acids by *K. intermedius* V-05.

Analysis of Effects Table			
	Estimated effect	**t value**	***p*-value**
$\mathbf{X_1}$	0.131557	2.3213	0.048819
$\mathbf{X_1^2}$	-0.438796	-7.3866	$7.718e^{-05}$
$\mathbf{X_2}$	0.060984	1.0761	0.313282
$\mathbf{X_2^2}$	-0.318196	-5.3565	0.000681
$\mathbf{X_3}$	0.101311	1.7876	0.111642
$\mathbf{X_3^2}$	-0.134596	-2.2658	0.053241
$\mathbf{X_1*X_2}$	-0.015000	-0.2033	0.843955
$\mathbf{X_1*X_3}$	0.005000	0.0678	0.947627
$\mathbf{X_2*X_3}$	-0.012500	-0.1694	0.869657
$\mathbf{R^2}$	0.9091		
Adjusted R^2	0.8069		

Analysis of Variance Table				
	SS*	**MS****	**F value**	***p*-value**
Linear (X_1, X_2, X_3)	0.42416	0.14139	3.2473	0.081209
Quadratic (X_1, X_2, X_3)	3.05777	1.01926	23.4102	0.000258
Interaction (X_1, X_2, X_3)	0.00325	0.00108	0.0249	0.994263
Residues	0.34831	0.04354		
Lack of adjustment	0.31141	0.06228	5.0636	0.106127
Pure error	0.03690	0.01230		

Legend: $\mathbf{X_1}$ – aspartic acid; $\mathbf{X_2}$ – phenylalanine; $\mathbf{X_3}$ – serine.

***** Sum of squares. ****** Mean square.