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Cyanide removal present in cassava wastewater (CWW): effects on the first stage of anaerobic digestion and complete anaerobic digestion

Florianópolis
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Orientador: Prof. Dr. Hugo Moreira Soares.
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O presente trabalho em nível de mestrado foi avaliado e aprovado por banca examinadora composta pelos seguintes membros:

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Prof. Hugo Moreira Soares, Dr.
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RESUMO

A mandioca é conhecida mundialmente como fonte de alimento em mais de 190 países. A indústria de mandioca gera quantidades significativas de resíduos líquidos e sólidos com elevada carga orgânica, amido e compostos tóxicos, como o cianeto (CN). Estima-se que para cada tonelada de mandioca processada, gera-se de 300 a 600 litros de efluente. Os glicosídeos cianogênicos (linamarina e lotaustralin) presentes na mandioca, sofrem hidrólise enzimática em contato com a linamarase, liberando HCN como produto final. Com isso, os resíduos gerados no processamento do tubérculo liberam para o meio ambiente compostos como CN livre ($CN^{-)}$ e ácido cianídrico (HCN) em alta concentração, além de glicosídeos, causando severo impacto ambiental, principalmente quando esses compostos se associam à elementos metálicos. Devido a ineficiência de remoção dos métodos físico-químicos quando há alta concentração de CN e complexos metálicos, os modelos biológicos são alternativa viável, além de outras vantagens, como geração de produtos de valor agregado. Na literatura vários microrganismos são descritos devido capacidade de tolerar e degradar CN utilizando-o como fonte de carbono e nitrogênio. Neste contexto, o presente estudo teve como objetivo a remoção do CN do efluente oriundo da indústria de farinha de mandioca, via processo biológico anaeróbio utilizando uma cultura mista proveniente de reator anaeróbio de fluxo ascendente (RAFA). Além disso, analisou o efeito do CN no reator acidogênico (RA), ou seja, com a inibição das metanogênicas por meio do BES, e na digestão anaeróbia completa (DAC) durante 13 dias. A eficiência de remoção de CN no primeiro dia foi >83% e >86% para RA e DAC, respectivamente. Entre o 2° e 6° dia, DAC obteve remoção ligeiramente superior ao RA, porém no 7° dia a eficiente de ambos fora acima de 99%. A produção de biogás na DAC foi afetada pelo pH e CN até o 5° dia. A partir do 2° dia, a produção acumulativa de biogás no RA foi constante, provavelmente devido a conversão da matéria orgânica complexa em biogás. A composição do biogás no RA (44% de H_2; 4% de CO_2; e 1% de CH_4) mostra que a maioria das metanogênicas foram inibidas; enquanto na DAC 71% da composição do biogás era metano. *Pseudomonas stutzeri*, conhecida como degradadora de CN, foi identificada no tempo inicial, diferentemente do tempo final em RA e DAC. No entanto, os gêneros *Clostridium* e *Pseudomonas* foram identificados no tempo final e inicial, catalogados como grupos tolerantes ao CN. Com isso, pode-se inferir que o RA e DAC removem CN concomitante a produção de produtos de valor agregado (biogás e AGVs), com período de aclimatação.

Palavras-chave: resíduos da indústria de mandioca; biodegradação de cianeto; digestão anaeróbia; glicosídeos cianogênicos.

ABSTRACT

Cassava is known worldwide as a food source, covering more than 190 countries. The cassava industry generates large amount of liquid and solid waste with a high organic load, starch, and toxic compounds such as cyanide (CN). It is estimated that for each ton of cassava processed, 300 to 600 L of effluent are generated. The cyanogenic glycosides (linamarin and lotaustralin) present in cassava undergo enzymatic hydrolysis in contact with linamarase, releasing HCN as a final product. As a result, the waste generated in tuber processing releases compounds such as free CN (CN-) and hydrocyanic acid (HCN) in high concentrations into the environment, in addition to glycosides, causing severe environmental impact, especially when these compounds are associated with elements metallic. Due to the inefficiency of removal of physical-chemical methods when there is a high CN concentration and metal complexes, biological models are a viable alternative, in addition to other advantages, such as the value-added products generation. In the literature several microorganisms are described due to their ability to tolerate and degrade CN using it as a carbon and nitrogen source. In this context, the present study aimed to remove CN from the effluent from the cassava flour industry, via an anaerobic biological process using a mixed culture from an Upflow Anaerobic Sludge Blanke (UASB). Furthermore, it analyzed the CN effect on the acidogenic reactor (AR) and on the complete anaerobic digestion (CAD) during 13 days. Day one CN removal efficiency was >83% and >86% for AR and CAD, respectively. Between the 2nd and 6th day, CAD achieved removal slightly higher than AR, but on the 7th day the efficiency of both was above 99 %. The biogas production in CAD was affected by pH and CN until the 5th day. From the 2nd day, the cumulative biogas production in the AR was constant, probably due to the conversion of complex organic matter into biogas. The biogas composition in AR (44% for H_2; 4% CO_2; and 1% CH_4) shows that most methanogens were inhibited; while in the CAD 71 % of the biogas composition was methane. *Pseudomonas stutzeri*, known as a CN degrader, was identified at the initial time, differently from the final time in AR and CAD. However, the genera *Clostridium* and *Pseudomonas* were identified at the final and initial times, cataloged as CN-tolerant groups. With this, it can be inferred that AR and CAD remove CN concomitantly with the production of value-added products, with an acclimatization period.

Keywords: cassava industry waste; cyanide biodegradation; anaerobic digestion; cyanogenic glycosides.

RESUMO EXPANDIDO

INTRODUÇÃO

A mandioca (*Manihot esculenta Crantz*), usualmente chamada de aipim ou macaxeira, também conhecida em outras localidades como "pão da terra"(ADAMS et al., 2009), é uma cultura que gera vários subprodutos que são utilizados na cozinha, como farinha, fécula, polvilho e tapioca (ADAMS et al., 2009; AMORIM et al., 2018). A cultura não exige cuidados acentuados enquanto ao seu manejo, podendo ser cultivada em diversas regiões, independente da precipitação, altas temperaturas, e baixa fertilidade nutricional do local (MODESTO JÚNIOR; ALVES, 2016; SALGAONKAR; MANI; BRAGANÇA, 2019).

O processamento de raízes de mandioca gera uma grande quantidade de poluição (i.e., emissões sólidas, líquidas e gasosas), e seu descarte direto pode causar problemas ambientais (FOONG et al., 2020; ISMANTO et al., 2010; OGHENEJOBOH et al., 2021; OLAOYE et al., 2020). Estima-se que uma tonelada de raiz de mandioca processada pode produzir de 300 a 600 L de águas residuais (DE CARVALHO et al., 2018; PAIXÃO et al., 2000). Esse efluente, também conhecido como *manipueira*, é um líquido amarelado caracterizado por alto teor de sólidos suspensos (lipídeos e carboidratos insolúveis) e cianeto (CN), substância tóxica que necessita de adequado gerenciamento (COUTINHO RODRIGUES et al., 2021; KAEWKANNETRA et al., 2009; VIANA; DÜSMAN; VICENTINI, 2014). O CN é produzido por plantas, animais e microrganismos como mecanismo de defesa (KJELDSEN, 1999; PARK; TREVOR SEWELL; BENEDIK, 2017; TERADA et al., 2022; TORKAMAN et al., 2021). Nesse contexto, as raízes da mandioca contêm glicosídeos cianogênicos (GLC) (i.e., linamarina e lotaustralina), que sofrem hidrólise enzimática em contato com a linamarase (*β-glicosidase*) e, consequentemente, liberam ácido cianídrico (HCN) como produto final (TORKAMAN et al., 2021; ZHONG et al., 2020).

Portanto, o processamento da mandioca libera GLC e compostos de CN no efluente, que devem ser tratados, evitando danos ao meio ambiente (CHISTÉ et al., 2010; COUTINHO RODRIGUES et al., 2021; LUCHESE; RODRIGUES; TESSARO, 2021). Em algumas indústria de mandioca, o efluente pode ultrapassar 200 $mgCN.L^{-1}$ (POTIVICHAYANON et al., 2020; SILLER; WINTER, 1998a, 1998b). Processos físico-químicos são utilizados para a degradação do CN, porém esses apresentam algumas desvantagens, como: ineficiência na remoção de altas concentrações de CN; eficaz apenas com CN livre e complexos de CN metálicos fracos; custos elevados; difícil manutenção; e produção de compostos tóxicos (ALVILLO-RIVERA et al., 2021; ESKANDARI et al., 2019; GUAMÁN GUADALIMA; NIETO MONTEROS, 2018; VEDULA; DALAL; MAJUMDER, 2013). Apesar do CN ser considerado um potencial inibidor, principalmente para as *archaeas* metanogênicas, há relatos na literatura de produção de biogás concomitante a remoção do CN por via anaeróbia com rendimento de 82 % de CH_4 e taxa de remoção do CN acima de 92% (GIJZEN; BERNAL; FERRER, 2000; GLANPRACHA et al., 2018; GLANPRACHA; ANNACHHATRE, 2016; ZAHER et al., 2006). No entanto, são inexistentes estudos avaliando a remoção de CN na digestão anaeróbia separadamente, ou seja, em reator acidogênico e de digestão anaeróbia completa. Neste contexto, este trabalho visou a avaliação da biodegração de CN em reator acidogênico e de digestão anaeróbia completa.

OBJETIVO

O presente estudo propõe-se avaliar a biodegradação do CN presente em efluente de mandioca em dois retores de digestão anaeróbia de estágio único, havendo a inibição das *arqueas* metanogênicas em reator (reator acidogênico). Para alcançar o foco do trabalho,

algumas medidas foram tomadas. Mais especificamente, foram determinadas as condições operacionais dos processos (pH, tempo de operação, eficiência de remoção dos poluentes) de acordo com a literatura. Além disso, promoveu-se a identificação dos microrganismos relacionados à degradação de CN, e análise da produção de produtos de valor agregado.

METODOLOGIA

Para realização dos ensaios o substrato enriquecido com CN foi proveniente da prensagem da mandioca de uma empresa produtora de farinha, localizada no município de Sangão, estado de Santa Catarina/Brasil. O inóculo utilizado foi o lodo granular anaeróbio do reator anaeróbio de fluxo ascendente (RAFA). Após coleta do material, seguiu-se para a caracterização dos mesmos. Para o ensaio experimental foram utilizados frascos de penicilina de 100 mL com *headspace* de 50 mL. A digestão anaeróbia foi realizada durante 13 dias com agitação orbital (120 rpm) e temperatura (35 ± 2°C) controlada. Resumidamente, a remoção de CN foi realizada em reatores descontínuos de digestão acidogênica (AR) e anaeróbia completa (CAD), utilizando água salina e inóculo anaeróbio como controle. A inibição das *archaeas* metanogênicas no reatores acidogênicos foi realizada com BES na concentração de 40 μmol·mL^{-1} (KOSSE; LÜBKEN; WICHERN, 2016). A concentração inicial de DQO (6.700 ± 49,6 mg·L^{-1}) e CN (3,85 ± 0,1 mg·L^{-1}) nos reatores foi ajustada para evitar a inibição dos microrganismos. A concentração de inóculo utilizada foi de 3,6 g·L$^{-1}_{VST}$, correspondendo a uma razão de 1,44 (5,20 g·L$^{-1}_{VSTsubstrato}$ / 3,6 g·L$^{-1}_{VSTinóculo}$) (WANG et al., 2015b). O pH foi ajustado para 8,0, minimizando a liberação de HCN na fase gasosa (DAS; SANTRA, 2011; MARTÍNKOVÁ et al., 2023). Os reatores eram operados em sacrifício, ou seja, a cada dia, 3 frascos de cada condição era retirado do ensaio para posterior análises. Os parâmetros medidos nos ensaios foram: concentração de CN, DQO, nitrogênio amoniacal, nitrato, variação de pH; produção de biogás e ácidos graxos voláteis (AGV). AGV, CN, DQO, pH, nitrogênio amoniacal, nitrato foram analisados diariamente durante o período de ensaio. A produção cumulativa de biogás foi analisada a cada 12 horas até o final do experimento. A determinação do CN foi realizada pela metodologia colorimétrica (DROCHIOIU, 2002b; SURLEVA; DROCHIOIU, 2013). A cada dia, as amostras eram retiradas, centrifugadas à 5000 g (4 °C) por 15 min, filtradas (poro de 0,22 μm) e analisadas. AGVs foram quantificados por cromatografia líquida de alta eficiência (HPLC); DQO, por meio do método de refluxo fechado; nitrato e nitrogênio amoniacal por método colorimétrico (APHA, 2005; JEONG; PARK; KIM, 2013). A composição do biogás foi realizada por meio da cromatografia gasosa (GC), utilizando detector de chama (FID) e de condutividade térmica (TCD) (DA SILVA et al., 2022). Foi utilizada a seringa de vidro esmerilhada para analisar a produção acumulativa de biogás. Todas a análises foram realizadas em ao menos três replicatas.

RESULTADOS E DISCUSSÃO

A caracterização do substrato (efluente de mandioca) mostra que o resíduo é fonte rica em nutrientes, matéria orgânica (81.730 ± 534 mg O_2·L^{-1}) e principalmente cianeto (139,75 ± 5,40 mg·L^{-1}). A análise de Carbono Orgânico Total (COT) e Nitrogênio Kjeldahl confirmou que a relação C/N (54) do resíduo é alta, havendo necessidade em suplementar com nitrogênio. As características apresentadas no presente estudo são corroboradas com a literatura, porém parâmetros como DQO e cianeto são divergentes de alguns estudos (KAEWKANNETRA; CHIWES; CHIU, 2011; POTIVICHAYANON et al., 2020; QUAN et al., 2014). A variação na concentração de DQO e CN é devido ao processo industrial, espécie de mandioca, região, *stress* hídrico (ADEMAKINWA; AGUNBIADE; FAGBOHUN, 2021; CARDOSO et al., 2005; RAY et al., 2015). A produção cumulativa de biogás foi semelhante ao RA e DAC nos três primeiros dias. A partir do 2º dia, o RA não produziu biogás,

provavelmente devido ao valor do pH e ao efeito inibitório do CN e BES sobre os metanogênicos. Além disso, no 1º dia, o pH no RA foi drasticamente reduzido devido à conversão da matéria orgânica em ácidos. Aparentemente, a ação de inibição do CN aos metanogênicos (DAC) ocorreu até o 5º dia, ao contrário do observado em outro estudo que demonstrou apenas 3 dias de inibição (GLANPRACHA et al., 2018). Ao analisar a composição do biogás do RA, a concentração de CH_4 foi <1% e H_2>44%, confirmando o efeito inibitório do BES sobre os metanogênicos. A concentração de CH_4 (i.e., 74%) encontrada na DAC foi semelhante à literatura (GLANPRACHA et al., 2018; LI et al., 2019; NOVAK et al., 2013). No entanto, em DAC a concentração de H_2 foi baixa (i.e., 0,54%), provavelmente devido à concentração de amônia no meio, uma vez que os metanogênicos hidrogenotróficos são mais tolerantes à amônia do que os metilotróficos e acetoclásticos (YENIGÜN; DEMIREL, 2013; YI et al., 2023). A análise da produção de AGV mostrou que o ácido acético foi o ácido majoritário, seguido do ácido butírico e propiônico, tanto no RA quanto no DAC. A concentração máxima alcançada para o ácido acético foi de 2,62 $g \cdot L^{-1}$, enquanto para o ácido butírico foi de 1,23 $g \cdot L^{-1}$ ambos no 13º dia. No RA, a DQOs teve um aumento considerável no primeiro dia. Continuando com um aumento até o terceiro dia e uma pequena queda no quarto. A DQOs no tempo inicial em ambos os reatores (RA e DAC) era de 6.700 ± 49,6 $mgO_2 \cdot L^{-1}$. No entanto, a eficiência de remoção no DAC foi de 91,78 %. Porém, a DQOs no RA aumentou para 10.643 ± 156 $mgO_2 \cdot L^{-1}$. O aumento na concentração de nitrogênio amoniacal em RA e DAC (166 e 170 $mg \cdot L^{-1}$, respectivamente) foi perceptível a partir do 3º dia.

A remoção de CN em ambos os reatores, RA e DAC, no primeiro dia foi de 83,26 e 86,73%, respectivamente. Do 1º ao 6º dia, a eficiência de remoção foi ligeiramente diferente entre os reatores. Nesse período, a concentração de CN a partir do 1º dia era de 0,64 $mg \cdot L^{-1}$, obtendo eficiência de remoção em 69,88 % até o 6º dia no RA. No entanto, para DAC, no mesmo período, a remoção de CN atingiu 97,27 %, i ndicando que os microrganismos metanogênicos podem degradar rapidamente o CN. Em ambos os reatores estudados, a análise microbiana identificou dois filos (*Arqueas* e Bacteria) no tempo inicial e final. Além disso, *Pseudomonas stutzeri* que possui capacidade de remover o cianeto, foi identificado no tempo inicial, mas não no tempo final, sugerindo que a geração de metabólitos por outros microrganismos pode ter afetado o crescimento. Analisando os experimentos realizados nos RA, *Clostridium butyricum* foi mais abundante quando comparado ao tempo inicial.

CONSIDERAÇÕES FINAIS

Os dados analisados permitiram observar que o resíduo de mandioca utilizado no presente estudo é uma fonte rica de carboidratos, cianeto e outras formas orgânicas. Por isso, é viável a aplicação de modelos biológicos a fim de converter esses poluentes pelos microrganismos em produtos menos tóxicos e geração de produtos de interesse. Também demonstrou que as características observadas nestes resíduos são variáveis na literatura, que está diretamente ligado a condições climáticas, região, espécie de mandioca, tipo de indústria. Muitos estudos demonstram bons resultados de eficiência de remoção de CN em poucos dias de tratamento, mas no presente estudo a remoção de CN foi acentuada já no 1º dia, chegando a >83%, tanto no processo acidogênico (RA) quanto na digestão completa (DAC). Os resultados demonstram que a remoção de CN do efluente de mandioca pode ser viável tanto em processos de digestão acidogênica quanto anaeróbia completa. A escolha do processo dependerá do produto de interesse a ser gerado e da tecnologia disponível para recuperação desses produtos finais. A concentração de AGV foi alta e constante nos RA devido à inibição de metanogênios. Maior rendimento na produção de ácido acético seguido do butírico. A produção cumulativa de biogás no DAC foi baixa por 5 dias, atingindo um ponto de inflexão no 11º dia. *Clostridium butyricum* e *C. quinni* foram mais abundantes em RA e DAC, respectivamente.

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LIST OF ABBREVIATIONS AND SYMBOLS

AD - Anaerobic digestion

AR – Acidogenic reactor

CAD – Complete anaerobic digestion

CN- Cyanide

CNGs - Cyanogenic glycosides

COD - Chemical oxygen demand

CPe – Cassava peel

CWW – Cassava wastewater

DFS - Dissolved Fixed Solids

DQO – Demanda Química de oxigênio

DTS - Dissolved Total Solids

DVS - Dissolved Volatile Solids

EC – Electric conductivity

FSS - Fixed Suspended Solids

KCN – Potassium cyanide

HRT - Hydraulic retention time

OLR - Organic loading rates

P - Pressure of the gas phase at the time of reading

$\mathbf{P_0}$ - Normal pressure

$\mathbf{P_W}$ - Vapor pressure of the water as function of the temperature of the ambient space

T - Temperature of the fermentation gas of the ambient space

$\mathbf{T_0}$ - Normal temperature

TFS - Total Fixed Solids

TS - Total Solids

TSS - Total Suspended Solids

TVS - Total Volatile Solids

UASB - Upflow Anaerobic Sludge Blanket reactor

V - Reactional volume

VAP – Value-added products

V_b - Volume of the gas as read off

VFA – Volatile fatty acids

V_N - Volume of the gas in the normal state

VSS - volatile suspended solids

VSS - Volatile Suspended Solids

SUMMARY

Chapter

1

Introduction

In this chapter, it is presented a brief introduction to the research developed, the problem and motivation, hypotheses, objectives, and a conceptual diagram.

1.1 INTRODUCTION

Cassava is present in more than 190 countries. In addition, Brazil, the Democratic Republic of Congo, Ghana, Indonesia and Thailand account for more than half of world cassava production (FAOSTAT, 2021). In Brazilian territory, the North, Northeast and South regions stand out in production terms (IBGE, 2022). Moreover, in the 2022 harvest, the state of Santa Catarina had a cassava production of 311,643 tons. Cassava is used by industry to manufacture food (ADAMS et al., 2009; FALADE; AKINGBALA, 2010). However, this processing generates a significant amount of liquid waste (LUCHESE; RODRIGUES; TESSARO, 2021; OLAOYE et al., 2020). In addition, the cassava processing industry is among those that most contribute to food waste in developing countries, reinforcing the importance of mitigating measures (DE JESUS et al., 2022).

It is estimated that flour industry produces from 0.28 $m^3 \cdot t^{-1}$ to 0.6 $m^3 \cdot t^{-1}$ of cassava wastewater (CWW) (ARAÚJO et al., 2014; DE CARVALHO et al., 2018). In addition, cassava roots contain cyanogenic glycosides CNG (e.g., linamarin and lotaustralin), which undergo enzymatic hydrolysis on contact with linamarase (β-glucosidase) and consequently release HCN as the end product (TORKAMAN et al., 2021; ZHONG et al., 2020). CNGs concentration determines the cyanogenic potential of the plant, that is, the ability of the plant to produce free cyanide compounds (PINTO ZEVALLOS; PEREIRA QUEROL; AMBROGI, 2018). The CN concentration present in CWW is quite variable. However, most studies in the literature report high concentrations, ranging from 86 to 252 $mgCN \cdot L^{-1}$ (ADEMAKINWA; AGUNBIADE; FAGBOHUN, 2021; GUSMÃO et al., 2018; KAEWKANNETRA; CHIWES; CHIU, 2011; NEVES et al., 2014; POTIVICHAYANON et al., 2020).

Physical-chemical and biological processes are used for CN degradation. However, the physical-chemical ones have disadvantages, such as inefficiency in removing high CN concentrations , effective only with free CN and weak metal CN complexes, high costs, difficult maintenance and production of toxic compounds (ALVILLO-RIVERA et al., 2021; ESKANDARI et al., 2019; GUAMÁN GUADALIMA; NIETO MONTEROS, 2018; VEDULA; DALAL; MAJUMDER, 2013). On the other hand, the microbial community from a biological process can use CN as a nutrient source or convert it to other non-toxic by-products (GUPTA; BALOMAJUMDER; AGARWAL, 2010; GUPTA; AHAMMAD; SREEKRISHNAN, 2016; LUQUE-ALMAGRO et al., 2018; PEREIRA; ARRABAÇA; AMARAL-COLLAÇO, 1996). Following what has been reported in the literature, the anaerobic biological process was applied to remove cyanide from CWW. However, it was

decided to separate the anaerobic digestion (AD) into two stages, hydrolysis, acetogenesis, acidogenesis (1) and complete AD (2), in order to compare which stage efficiently removes cyanide. In addition, there are no studies reports using cassava effluent enriched with cyanide, aiming at removal in the acidogenic phase.

1.2 MOTIVATION AND INNOVATION

In recent years, there has been a growing concern about reducing the waste amount produced. Faced with this problem, a new concept arises of recovering and using these residues to obtain products of interest, such as methane, hydrogen, and fertilizers. The biorefinery concept has also gained space, mainly due to the search for technologies that reduce and replace greenhouse gases. Given this, other alternatives for the reduction of pollutants concomitant with the value-added products generation are appreciated. Therefore, using waste from a widespread worldwide industry has potential, especially if this industry generates large waste amounts. However, the literature did not show studies analyzing the cyanide degradation present in cassava using acidogenic and complete anaerobic digestion reactors. On the other hand, studies evaluating the cyanide influence on methanogens are well reported. In addition, few studies evaluate cyanide removal with the production of hydrogen and VFA, mainly using cassava effluent.

1.3 HYPOTHESES

The present dissertation is based from some hypotheses that will be answered in the experiments. These are described below:

- Cyanide degradation in acidogenic and complete anaerobic digestion reactors occurs with good removal efficiency;
- The cumulative biogas production will be affected by the cyanide presence;
- The organic matter concentration in CWW will impact the obtation of value-added products.

1.4 OBJECTIVES

1.4.1 General objective

The master thesis proposes to evaluate the cyanide removal from cassava processing waste through anaerobic digestion in an acidogenic and methanogenic phase reactor to remove CN and produce alternative energy sources concomitantly.

1.4.2 Specific objectives

The specific objectives are related to each stage of the present study, that is, literature review and bench assay. Therefore, below are the specific objectives guided for this dissertation:

Specific objectives to chapter 3 (literature review)

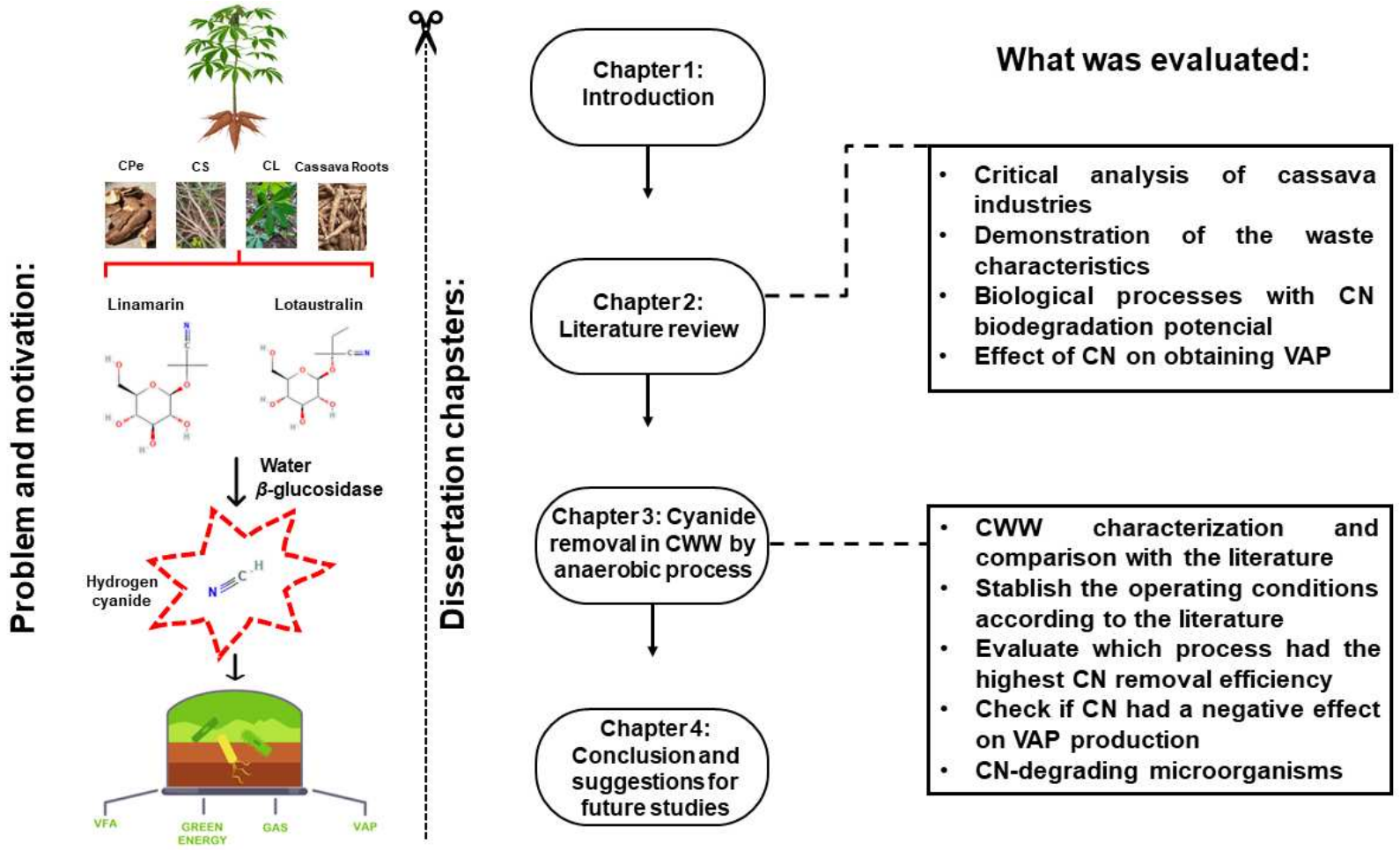
- Literature survey of studies related to the cyanide removal in cassava effluent;
- Cassava processing industries identification;
- Find out the characteristics cassava industrial waste;
- Point out the biological processes and microorganisms capable of cyanide degrading and/or tolerating;
- Suggest trends for more optimized processes that remove cyanide and generate value-added products.

Specific objectives to chapter 4 (banch experiments)

- Characterize the CWW and compare with the literature;
- Evaluate the efficiency of AR and CAD processes on CN removal;
- Verify the CN effect on VAP production;
- Determine the population and relative abundance of microbial community regarding the CN degradation and/or tolerance.

1.5 CONCEPTUAL DIAGRAM

Figure 01 - Conceptual diagram of the master thesis showing the problem and motivation, thesis structure, and what was evaluated in the experimental and review chapter



Source: From the author.

Chapter 2

Literature review

In this chapter, a brief review of the literature presents the relevant issues for this research. It also covers the most used methods to cyanide determine, cyanogenic glycosides conversion in cyanide, and enzymatic pathways of cyanide degradation. In addition, this chapter is in the final stage of journal submission.

2 LITERATURE REVIEW: BIOLOGICAL CYANIDE REMOVAL PRESENT IN CASSAVA WASTE: A REVIEW

Abstract

Cassava is a food used to manufacture various products (e.g., flour, ethanol, sago, tapioca) used worldwide. From the harvest to the processing of this tuber, large amounts of liquid and solid waste are generated with a significant of organic matter and cyanide (CN). The characteristics of cassava waste are well-documented, but some divergent parameters are due to climatic factors, variety of species, and type of industry. The CN quantification in cassava waste is performed with different methodologies, but the direct methods based on ninhydrin are simple, fast, and highly selective. Under anaerobic and aerobic conditions cyanide is degraded and transformed into by-products. Optimization of operating conditions, such as temperature, pH, and microorganisms acclimatization, is crucial to prevent inhibition and low yield of biofuels. Granular brewer inoculum is a viable alternative to CN remove due to a combination of simultaneous abiotic and biotic processes. In addition, there are microorganisms such as *S. cerevisiae* capable of CN tolerating and degrading. Biological processes are well established for cyanide removal, but other alternatives such as electro-biodegradation, cyanide-degrading enzymes immobilization, microbial fuel cell (MFC), and two-step anaerobic digestion (AD), gain prominence due to efficiency and obtaining value-added products (VAP).

Keywords: cassava waste, cyanide biodegradation, cassava industry, manipueira, cyanogenic glycosides, cassava wastewater

2.1 INTRODUCTION

Cassava or *manioc* (*Manihot esculenta* Crantz) is a shrub belonging to the *Euphorbiaceae* family, which contains considerable amounts of starch, protein and other nutrients in its roots (ASSANVO et al., 2017; BARCELOUX, 2009; FALADE; AKINGBALA, 2010; LATIF et al., 2019). Therefore, it is an essential crop in more than ninety-nine countries, playing an important socio-economic role in rural areas, especially in developing countries, and which applies to the economic segments (FAOSTAT, 2021; SANTOS et al., 2020; WATTHIER et al., 2019). Brazil, Democratic Republic of the Congo, Ghana, Indonesia, and Thailand represent more than half of worldwide cassava production, totaling 188×10^6 tons in 2020 (FAOSTAT, 2021).

The processing of cassava roots produces a large amount of pollution (e.g., solid, liquid, and gaseous emissions), and their direct disposal can cause environmental problems (FOONG et al., 2020; ISMANTO et al., 2010; OGHENEJOBOH et al., 2021; OLAOYE et al., 2020). One ton of processed cassava root can produce from 300 to 600 L of wastewater (DE CARVALHO et al., 2018; PAIXÃO et al., 2000). Other authors report the unit of mass being 250 to 600 kg of cassava wastewater (CWW) for each ton of processed cassava (OGHENEJOBOH et al., 2021). The CWW, also known as *manipueira*, is a yellowish liquid characterized by highly suspended solids (e.g., lipids and non-soluble carbohydrates) and cyanide, which is a toxic substance that needs the correct waste management (COUTINHO RODRIGUES et al., 2021; KAEWKANNETRA et al., 2009; VIANA; DÜSMAN; VICENTINI, 2014).

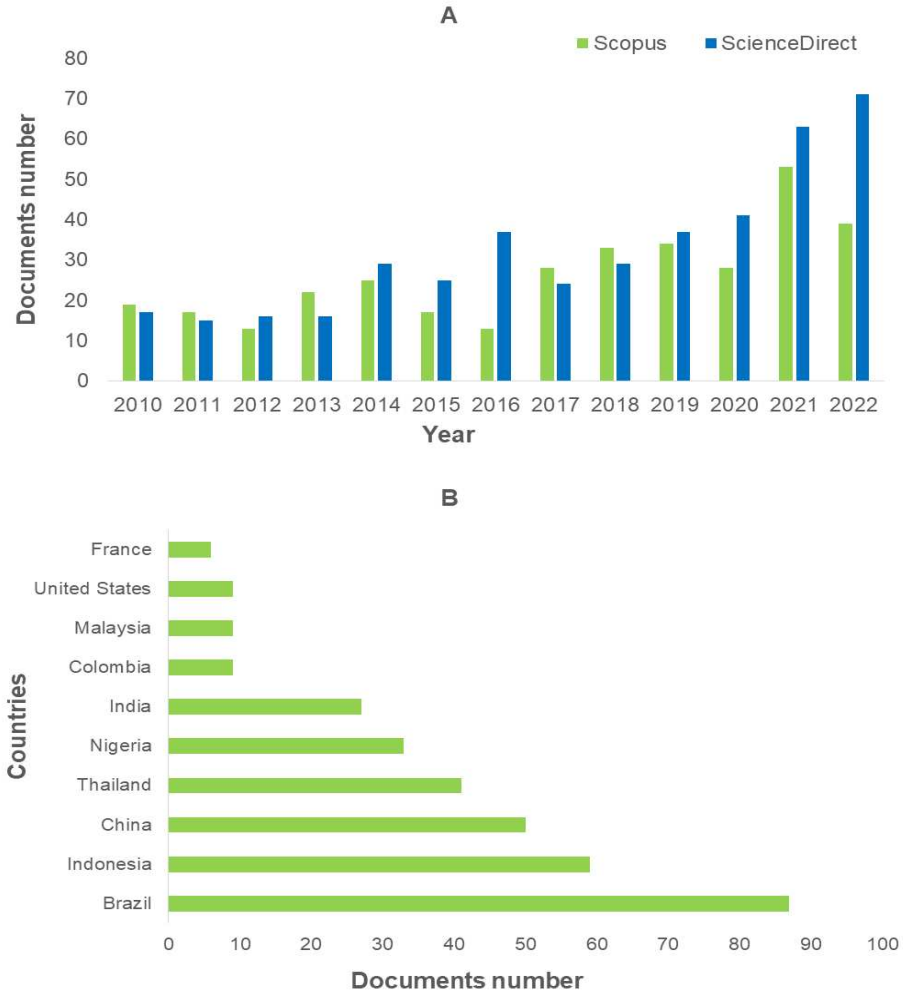
The cyanide (CN) is produced by plants, animals, and microorganisms as a defense mechanism (KJELDSEN, 1999; PARK; TREVOR SEWELL; BENEDIK, 2017; TERADA et al., 2022; TORKAMAN et al., 2021). The CN binds to metals, making metalloenzymes, such as cytochrome-c oxidase (Cox), unusable, resulting in impaired cell respiration (DAS et al., 2021; DENG et al., 2010; KOKSUNAN et al., 2013; LUQUE-ALMAGRO; MORENO-VIVIÁN; ROLDÁN, 2016; SURLEVA et al., 2016). The exposition of humans to 1 $mg \cdot kg^{-1}$ per body weight of hydrogen cyanide gas (-HCN) can result in seizures and cardiac or cardiorespiratory arrest. Furthermore, higher doses are lethal for humans (PARK; TREVOR SEWELL; BENEDIK, 2017; POTIVICHAYANON et al., 2020; RAYBUCK, 1992; SILLER; WINTER, 1998a).

The amount of CN found in cassava differs between species and can range among 0.075 and 1 gCN per kg of root (KAEWKANNETRA et al., 2009). In CWW, the CN concentrations have been usually reported ranging from 26 to 89 $mg \cdot L^{-1}$; however, this value can reach more than 200 $mg \cdot L^{-1}$ depending on the root processing (CHATURVEDI; VERMA, 2016; COUTINHO RODRIGUES et al., 2021; KAEWKANNETRA; CHIWES; CHIU, 2011; POTIVICHAYANON et al., 2020). Therefore, wastewater cannot be released into water bodies without proper treatment (ZHANG et al., 2019). CN is released as hydrogen cyanide (-HCN) when cyanogenic glycosides (CNGs) in cassava are hydrolyzed (APEH et al., 2021; OLIVEIRA; REIS; NOZAKI, 2001b).

The literature survey was carried out in the Scopus database (**Figure 02-A**) from 2010 to 2022 with the keywords “biodegradation” OR “treatment” AND “cassava waste”; the search returned the following results within the search area: (77.4 %) are articles, 40

(11.7 %) conference paper, 19 (5.0 %) are reviews, 17 (3.5 %) conference review, and 8 (2.3 %) are book chapters. According to **Figure 02-A**, the period from 2014 to 2016 declined, but in recent years there has been an increase due to new research. The **Figure 02-B** presents Brazil first in the research area, probably due to being among the world's largest cassava producers. In the ScienceDirect database **Figure 02-A** with keywords "cyanide" AND "removal" AND "cassava" for the period 2010 to 2022 found 373 documents, being that 143 (37.34 %) research articles, 123 (33.97 %) book chapters, 92 (24.39 %) reviews, and 15 (4.02 %) other. In addition, as previously described, in the last five years, there has been an increase in research in the area, which can be explained by the adoption of the concept of bioindustry in cassava waste due to their potential to obtain by-products (DÍAZ et al., 2020; LUCHESE; RODRIGUES; TESSARO, 2021).

Figure 02 - Documents from Scopus Science Direct database



Footnote: documents by (A) year from Scopus database, with the keywords "biodegradation" OR "treatment" AND "cassava waste"; and Science Direct database with keywords "cyanide" AND "removal" AND "cassava". Countries with the highest number of searches (B) in the Scopus database with the keywords "biodegradation" OR "treatment" AND "cassava waste". The survey in the literature took place in December 2022.

Due to the large volume of waste containing CN generated in the cassava processing industry, it is necessary to direct biological treatment strategies that contribute to the green economy. This review discussed the stages of waste generation in the cassava industry, their physicochemical characteristics, methodologies for CN quantification, the CN removal present in cassava processing waste addressed by biological processes and optimal operating conditions (pH, temperature, retention time, and C/N ratio). In addition, it was critically discussed how the presence of CN could interfere with the obtention of the value-added products (VAP) by the biological treatment processes. To our knowledge, this is the first review paper reporting the CN removal from cassava waste by biological processes.

2.2 CASSAVA TRANSFORMATION PROCESS AND WASTE GENERATION

During harvest and transport, cassava is characterized by a significant loss of raw material (around 25%), making storage the most considerable challenge (FAO, 2020, p. 51; SIVAMANI et al., 2018). Cassava solid waste (CSW) is found in many forms: cassava bagasse (CB) or pulp (CP), peel (CPe), stem (CSt), rhizome (CR), leaf (CL), and periderm (FOONG et al., 2020; MURATA et al., 2021; OGHENEJOBOH, 2015). As previously mentioned, the cassava industry generates a high amount of solid and liquid waste, containing high amounts of carbohydrates and CN (COUTINHO RODRIGUES et al., 2021; LEAÑO; BABEL, 2012; LUCHESE; RODRIGUES; TESSARO, 2021; OGHENEJOBOH et al., 2021). However, the physics-chemical characteristics and CN levels depend on the region where the cassava was cultivated, processing chain and other intrinsic factors.

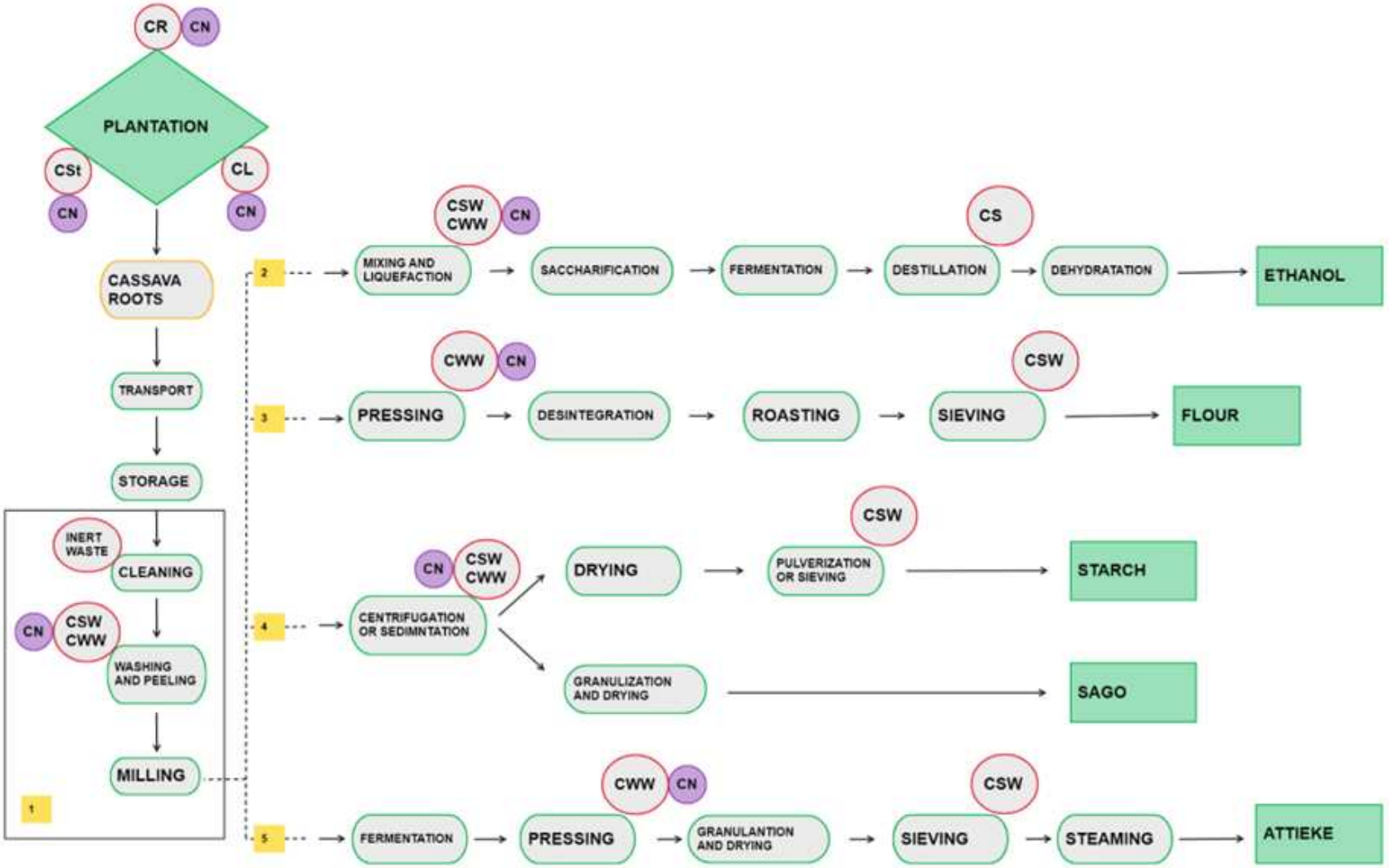
The production of cassava waste (**Figure 03**) starts with the cassava harvest disposal of leaves, stems, and rhizomes (SIVAMANI et al., 2018). Usually, the aerial parts (leaves) are put away as agricultural waste due to harvest logistics or intended for ruminant feed (FERNANDES et al., 2016; PEREIRA et al., 2016, 2018). In the beneficiation process (1), the inert waste (e.g., sand) and CSW (e.g., leaves, rhizome, stem) have little significance about the raw material (cassava roots). However, a large amount of wastewater is generated due to the considerable amount of water required in the process (CHAVALPARIT; ONGWANDEE, 2009). The **Figure 03** presents the cassava industrial processing flow diagram showing the waste (liquid and solid) generated from each step to produce ethanol, flour, starch, sago, and attieke. The CWW

and CP are the leading waste found in processing containing a high amount of organic carbon (COUTINHO RODRIGUES et al., 2021; GLANPRACHA et al., 2018; GLANPRACHA; ANNACHHATRE, 2016). Cassava peeling and washing produce wastewater with low organic content (COLIN et al., 2007; DE CARVALHO et al., 2018; THANGAVELU et al., 2020). Therefore, there are several sources of wastewater in the cassava industry, with low or high organic matter content.

During cleaning (1), inert residues (e.g., sand) are separated through a sieving process. Then, the roots are washed, peeled, and crushed, generating solid and liquid wastes containing CN and another toxic compound (DOS SANTOS et al., 2018; MODESTO JÚNIOR; ALVES, 2016, p. 214). A simple process with few steps follows flour production (3). The flour can be divided into dry and wet, the only difference being that the latter ferments (MAPA, 2011). In the pressing step, a yellowish CWW containing CN is generated (CHISTÉ et al., 2010; COUTINHO RODRIGUES et al., 2021). In the milling and pressing process, cyanogenic glycosides (CNGs) are decreased to acceptable concentrations in the final product (AYETIGBO et al., 2021).

Cassava can be used for ethanol production (2) due to the high amount of starch, consequently generating effluent with a high organic load (ZHANG et al., 2018a, 2018b). The CN release occurs in the initial peeling stages (1) when ethanol is produced (MURATA et al., 2021). For each ton of ethanol produced, 3.3 tons of cassava are required, and approximately 8 to 12 tons of wastewater are generated (ZHANG et al., 2016, 2010). The ethanol production process also creates cassava stillage (CS), which contains a high organic matter concentration (around Total COD 2,145 ± 20 $mg_{COD} \cdot g_{VS}^{-1}$) and suspended solids (WANG et al., 2012; YANG et al., 2017).

Figure 03 - Cassava processing in the industry



Footnote: Outputs (i.e., CR – Cassava Rhizome; CSt – Cassava Stem; CL – Cassava Leaves Inert Waste; CSW – Cassava Solid Waste; CWW – Cassava Wastewater; CN – Cyanide; CS – Cassava Stillage). Source: From the author

The production of starch or sago (4), generates wastewater up to 200 $mg \cdot L^{-1}$ of CN (DOS SANTOS et al., 2018; LUCHESE; RODRIGUES; TESSARO, 2021; LUQUE-ALMAGRO et al., 2018; SARAJAR; RAMADHANIA; PURWANTO, 2018). For each ton of cassava processed for sago production, 2 m^3 of wastewater is generated. The starch extraction can produce among 15 to 30 m^3 of wastewater for each ton of roots, depending on the process (FETTIG et al., 2013; KANDASAMY; BALACHANDAR; KUMAR, 2014; THANGAVELU et al., 2021). The effluent is mainly composed of starch and fibers, which gives a high load of organic matter (COD around 10,496 $mg \cdot L^{-1}$) (LEAÑO; BABEL, 2012; LUCHESE; RODRIGUES; TESSARO, 2021; SUN et al., 2012). According to Andrade et al. (2017), CWW represents approximately 30% ($w \cdot w^{-1}$) of the residues generated in the cassava starch industry. CB and CP are obtained from the processing of the tubercle to produce starch (KHANPANUEK et al., 2022; MARTINEZ et al., 2018; SIVAMANI et al., 2018).

The fermentation of bitter cassava roots produces Attieke or Attiéké (yucca flour). It is a steamed granular product, like couscous (AKELY; AZOUMA; AMANI, 2010; ASSANVO et al., 2017; FALADE; AKINGBALA, 2010). In the production process (5), the roots are peeled, cut, washed, and grated. After the fermentation (around 36 hours), the material is pressed, where CWW rich in CN is extracted, and then granules are obtained by removing fibers and residues (COULIN et al., 2006).

Therefore, the cassava industry generates several liquids and solid wastes with characteristics varying according to the production chain and the final product. Furthermore, some processes generate wastewater rich in cyanogenic compounds, showing an elevated environmental risk for the health of humans and animals.

2.2.1 Cyanogenic glycosides (CNGs) of cassava

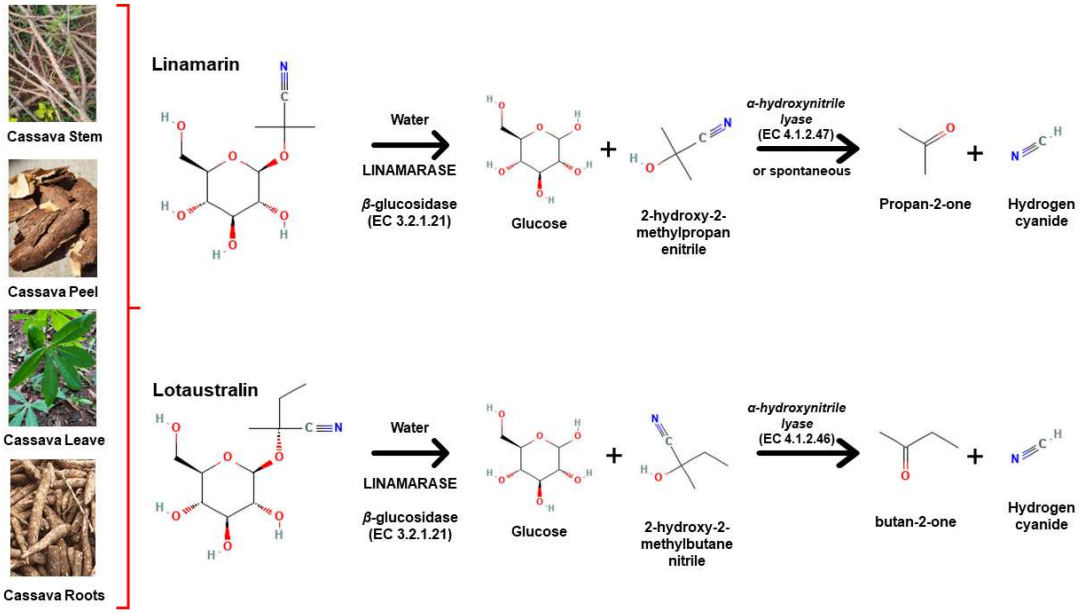
Composed of α-hydroxynitrile aglycone and a sugar moiety, CNGs are secondary plant metabolites (BARCELOUX, 2009). Around fifty types of CNGs are known and found in several plants. Nevertheless, only linamarin and lotaustralin are found in cassava (**Figure 04**), in a proportion of 95% and 5%, respectively (MONTAGNAC; DAVIS; TANUMIHARDJO, 2009; MOSAYYEBI et al., 2020; SIRITUNGA; SAYRE, 2003). The distribution of the CNGs is not well defined in the cassava plant tissues. However, it is found in higher concentrations in the roots, peels,

cortex, and leaf (DÓREA, 2004; FALADE; AKINGBALA, 2010; JØRGENSEN et al., 2011; ZHONG et al., 2021).

The CNGs present in CSW (e.g., CL, CP, and CPe) release hydrocyanic acid (HCN) and cyanohydrins upon contact with *β*-glucosidase (linamarase) as a result of enzymatic hydrolysis (**Figure 04**) (DÓREA, 2004; TORKAMAN et al., 2021; ZHONG et al., 2020). Cassava is classified as bitter or sweet based on total CN concentration. The sweet cassava presents an amount of <50 mg·HCN·Kg^{-1} of fresh roots, meanwhile, the bitter cassava has >100 mg·HCN·Kg^{-1} of fresh roots (CARDOSO et al., 2005; LEGUIZAMÓN et al., 2021). The presence of CNGs and the release of CN compounds give cassava industry waste a potential environmental risk. The linamarin and lotaustralin release CN by the linamarase hydrolysis (**Figure 04**), and this is readily soluble in water (CHISTÉ et al., 2010; DENG et al., 2010; GLANPRACHA et al., 2018; MODESTO JUNIOR; CHISTÉ; PENA, 2019; NWOKORO, 2016; QIN et al., 2021). According to **Figure 04**, after linamarin is hydrolyzed into glucose and acetone cyanohydrin, it subsequently degrades into acetone and CN, either spontaneously or by the action *α*-hydroxynitrile lyase. On the other hand, lotaustralin is cleaved by linamarase into glucose and C_5H_9NO, generating as final products butan-2-one and HCN (FALADE; AKINGBALA, 2010).

Strategies to remove the CN from cassava products are well-known in processing food (NWOKORO, 2016). Thermal treatments are extensively used (e.g., oven-drying and cooking) and can efficiently decrease the CN concentration (LAMBRI; FUMI, 2014; MODESTO JUNIOR; CHISTÉ; PENA, 2019). However, these traditional methods might cause a considerable loss of vitamins and amino acids (BRADBURY; DENTON, 2014). On the other hand, fermentation with *S. cerevisiae* promotes significant CNGs reduction without compromising these nutrients (LAMBRI; FUMI, 2014). The CNGs degradation can also be done by fermentation processes followed by enzymatic preparation (WU et al., 2012).

Figure 04 - Conversion pathway of linamarin and lotaustralin to cyanide by linamarase



Source: From the author.

2.2.2 Determination of CN in cassava samples

Cyanide (CN) can be found in four forms: i) total cyanide, ii) strong-metal cyanide, iii) weak and moderately strong metal-cyanide, and iv) free cyanide (KUYUCAK; AKCIL, 2013; MAHENDRAN et al., 2020; MONDAL; SARKAR; NAIR, 2021; OMOTOSHO; AMORI, 2015). CN is a generic term referring to all compounds with a triple bond between carbon and nitrogen (KJELDSEN, 1999). The free CN is associated with the sum of CN ion (CN^-) and HCN (KOKSUNAN et al., 2013). The sum of hydrocyanic acid and CN ions is considered free CN (GLANPRACHA et al., 2018; SURLEVA et al., 2016). CNGs are usually expressed as HCN due to limitations in the measurement methods (ZHONG et al., 2021). HCN is a colorless liquid having the distinctive odor of bitter almonds, with a vapor pressure of 84,000 Pa at 20 °C and miscible in water, being the deadliest form among the CN compounds mentioned above (DASH; GAUR; BALOMAJUMDER, 2009; KJELDSEN, 1999; RAYBUCK, 1992).

There are many different methods of CN quantification (e.g., colorimetric, titrimetric, ion chromatography, selective electrode), and each one has advantages and disadvantages (APHA, 2005; CENGIZ et al., 2015; CHRISTISON; ROHRER, 2007; COOKE, 1978; DROCHIOIU, 2002a; ESSERS et al., 1993; FISHER; BROWN, 1952; JASZCZAK et al., 2017; LAMBERT; RAMASAMY; PAUKSTELIS, 1975; SOUTO et al., 2021; SURLEVA; DROCHIOIU, 2013). One of the first studies that measured CN

concentration in cassava effluent used a colorimetric method capable of detecting HCN, CN^- and CN^- complexes (SILLER; WINTER, 1998a, 1998b). Torkaman et al. (2021) evaluated five different methodologies to detect CN in liquid samples. It was observed that colorimetric, titration, and ion-specific electrode methods, had similar and consistent results. However, data obtained by colorimetric assessment proved a fast and reliable method. Therefore, direct, indirect, and enzymatic reactions are used in colorimetric methods for CN quantification (HASSAN; HAMZA; KELANY, 2007). Some methods mentioned require many steps, in addition to using several reagents or toxic substances, such as pyridine and picric acid (ESSERS et al., 1993; FISHER; BROWN, 1952; LAMBERT; RAMASAMY; PAUKSTELIS, 1975). However, methods based on ninhydrin (2,2-dihydroxy-1,3-indanedione) are described as simple, fast, highly selective (i.e., >10 $ngCN \cdot mL^{-1}$), and capable of detecting CN directly from CWW samples (ADEMAKINWA; AGUNBIADE; FAGBOHUN, 2021; DROCHIOIU, 2002b; SURLEVA et al., 2016). Due to the solubility of CNGs in water, the method developed by Zhong et al. (2020) can express the actual condition of CN present in CWW, besides quantifying the CNGs in solid waste (e.g., CPe and CL).

2.2.3 Physics-chemical characteristics of cassava waste

The knowledge of the intrinsic characteristics of each waste generated from the cassava industry is crucial for decision-making because of its significant variability according to the process (OGHENEJOBOH et al., 2021). CSW (e.g., peel, bagasse, pulp) might cause an environmental impact on the soil or/and water body when mishandled. These residues have high starch content, and the C/N ratio showed by them (especially in CPe) may induce excessive acid production, lowering the soil pH. In addition, it can be released CN due to the presence of CNGs (CUZIN et al., 1992; MAKINDE; SALAU, 2017; NGUEFACK et al., 2022).

Adeyemo et al. (2014) found a CN concentration of around 7.4 $mg \cdot Kg^{-1}$ in CPe. Nevertheless, the adopted pretreatment in the study may have eliminated part of the CN by evaporation, affecting the original characteristic. The characteristics of CSW are presented in **Table 01.** CSW (e.g., CPe, CB/CP) are starch-lignocellulosic biomasses because 60 % ($w \cdot w^{-1}$) of its dry mass contains starch, being a promising alternative source of raw materials for biofuels and other VAP (MURATA et al., 2021; VIRUNANON et al., 2013). The different values can reinforce that the traits are linked to the cultivar,

process, and producing region. In literature, CP is also known as CB. Therefore, the normalization of the acronyms for the waste cassava must be carried out. The CB/CP is mainly composed of starch, cellulose, hemicellulose, lignin, and ash, among other minor compounds, containing a high percentage of moisture from 60 to 70%, and two CN forms: i) cyanohydrins ii) and HCN (DÍAZ et al., 2020; GLANPRACHA; ANNACHHATRE, 2016; PANICHNUMSIN et al., 2010).

Furthermore, as previously described, CNGs present in CP can be hydrolyzed and release CN compounds. Although, Glanpracha and Annachhatre (2016) demonstrated that it is possible to perform co-digestion using CP to obtain biofuel. Different CN amounts are found when analyzing different species of cassava. For example, total HCN and free HCN in leaf was studied by Modesto Junior et al. (2019) in nine varieties, finding values for total HCN and free HCN among 90.6 to 560.0 and 2.8 to 10.1 $mg \cdot Kg^{-1}$ (wet basis), respectively. The results demonstrate that CL contains high amounts of CNGs, requiring proper waste management. On the other hand, as presented in **Table 01**, the CN is not usually quantified for CP/CB and CPe. However, these wastes contain CNGs, which hydrolyzed release HCN or CN^-. The composition of CL (in terms of hemicellulose, cellulose, and lignin) showed no difference between the three cassava species described in **Table 01** (LEGUIZAMÓN et al., 2021). However, the values shown are similar to the other CSW presented in **Table 01**. In addition, to solid waste in the cassava industry, liquid waste can generate by-products due to its high organic load.

Table 01 - Chemical composition of cassava pulp/bagasse (CP/CB), cassava peel (CPe), and cassava leave (CL) found in the literature.

Cassava Waste	**Parameter**						**Reference**
	Cellulose	Hemicellulose	Lignin	Ash	Starch	Total CN ($mg \cdot Kg^{-1}$)	
Cassava Bagasse	12 %	16 %	9 %	2 %	NR	NR	(DÍAZ et al., 2020)
Cassava Leave	NR	NR	NR	4.68 – 7.06 $g \cdot 100g^{-1}$ DM	NR	91 - 561	(MODESTO JUNIOR; CHISTÉ; PENA, 2019)[a]
Cassava Leave	12 %[b] 11.5 %[c] 12.36 %[d]	48.8 %[b] 45.43 %[c] 56.73 %[d]	23.87 %[b] 30.23 %[c] 25.45 %[d]	15.62 %[b] 12.68 %[c] ND	NR	NR	(LEGUIZAMÓN et al., 2021)
Cassava Peel	NR	NR	1.92 ± 0.07 % DM	6.30 ± 0.34 % DM	46.16 ± 3.19 % DM	9.30 ± 0.42	(BAYITSE et al., 2015)
Cassava Peel	23.9 ± 0.9 %	9.4 ± 0.8 %	24.0 ± 1.1 %	7.4 ± 0.2 %	28.0 ± 1.4 %	NR	(ONA; HALLING; BALLESTEROS, 2019)
Cassava Peel	14.1 % DM	40.2 % DM	13.6 % DM	8.3 ± 0.4 % DM	NR	NR	(AHOU et al., 2021)
Cassava Peel	29.5 ± 5.25 %	35.65 ± 0.50 %	20.9 ± 0.99 %	5 ± 3 %	NR	NR	(AWOYALE; LOKHAT, 2021)
Cassava Peel	5.5-15 %	41.0-65 %	9.0-16 %	1.93-4.36 %	NR	NR	(KAYIWA et al., 2021)
Cassava Peel	NR	NR	NR	NR	50.91 ± 5.29 %	370	(MURATA et al., 2021)
Cassava Pulp	16.5 %	11.6 %	4.0 %	1.6 %	65.4 %	NR	(PANICHNUMSIN et al., 2010)
Cassava Pulp	4.11 % DM	4.20 % DM	1.15 % DM	11.9 % DM	75.1 % DM	NR	(VIRUNANON et al., 2013)
Cassava Pulp	NR	NR	NR	2.83 %	NR	3.26	(OKRATHOK et al., 2018)

Footnote: NR - not report; ND – not detected; DM - dry matter; [a] - values referring to nine varieties; [b,c,d] - values referring to three varieties: Mico, Pomberí and Campeona, respectively.

The BOD_5/COD ratio for some characterizations is among 0.25-0.8 (**Table 02**), which allows treatment by biological processes due to the CWW biodegradability (CHUN; YIZHONG, 1999; KAEWKANNETRA et al., 2009). However, a few studies point out ratios under 0.3 (see **Table 02**), producing an effluent with low biodegradability. This variability can occur due to industry technology level and the variety of cassava processed (HASAN et al., 2015). Moreover, the high content of organic matter present in the CWW makes it possible to use it to obtain VAP. The CWW wastewater physical-chemical characteristic found in the literature is presented in **Table 02**. CWW has acidic pH and, if released in the soil, can cause a reduction in the urease and dehydrogenase enzymatic activity since they are sensitive to pH variation (MIERZWA-HERSZTEK; GONDEK; BARAN, 2016).

Another characteristic with polluting potential and harmful to health is the CN. The free CN concentration in CWW showed an average of $267 \pm 80\ mg \cdot L^{-1}$, demonstrating its polluting potential (TORKAMAN et al., 2021). The variation of CN concentration found in the literature might be justified by the industrial process (i.e., to obtain starch or flour), variety of cassava species, country, or if there is water stress, a potentiating factor for increasing the CN content (ADEMAKINWA; AGUNBIADE; FAGBOHUN, 2021; CARDOSO et al., 2005). The processes of wastewater management (e.g., the junction of the washing water and the *manipueira*) in the industry can also change the composition (HASAN et al., 2015).

Table 02– CWW Physical-chemical characteristics.

Feedstock from	Characteristics								Reference
	COD ($mgO_2 \cdot L^{-1}$)	BOD ($mgO_2 \cdot L^{-1}$)	BOD_5/COD ratio	pH	Free Cyanide ($mg \cdot L^{-1}$)	TS ($mg \cdot L^{-1}$)	DS ($mg \cdot L^{-1}$)	SS ($mg \cdot L^{-1}$)	
Cassava alcohol wastewater	45,000 ± 1000	18,000 ± 500	0.4	4.5	86 ± 16	25,000 ± 550	NR	NR	(QUAN et al., 2014)
Cassava flour	79,487.12 – 92,441.79	NR	NR	5.11 – 6.24	26.14 – 89.89	NR	60 - 70 $mg \cdot g^{-1}$	NR	(Coutinho-Rodrigues et al., 2021)
Cassava mill wastewater	16,000 ± 562	NR	NR	5.5 ± 0.2	86 ± 2	NR	NR	3000 ± 562	(KAEWKANNETRA; CHIWES; CHIU, 2011)
Cassava mill wastewater	8,865	11.32	0,001	4.0	1.9	0.74 %	NR	NR	(HASAN et al., 2015)
Cassava mill wastewater	16,266.67-26,666	NR	NR	4.3-5.1	132.92-252.66	NR	NR	NR	(POTIVICHAYANON et al., 2020)
Cassava mill wastewater	10,000 ± 763	NR	NR	5.0 ± 1.0	102 ± 12	NR	1,800 ± 23	NR	(ADEMAKINWA; AGUNBIADE; FAGBOHUN, 2021)
Cassava starch wastewater	10,496	6,300	0.6	4.50-4.92	2.3	NR	NR	827	(SUN et al., 2012)
Gari processing wastewater	716 ± 2.08	385 ± 0.58	0.54	6.5 – 8.5	31.87	NR	NR	NR	(ODUGBOSE et al., 2020)
Sago wastewater	70,670 ± 60	5,040 ± 1	0,07	4.67 ± 0.03	4.46	4,570 ± 10	4,160 ± 20	NR	(THANGAVELU et al., 2021)
Tapioca industry	6,370.4	1,702.10	0.27	5.8	4.16	NR	NR	206.6	(SUHARTINI; HIDAYAT; ROSALIANA, 2013)

Footnote: NR: not reported; COD: chemical oxygen demand; BOD: biochemical oxygen demand; TS: total solids; DS: dissolved solids; SS: suspended solids.

The composition (e.g., high content of starch-lignocellulosic, high carbon content) of cassava wastes shows an enormous potential for obtaining biofuels, biohydrogen, biogas, biochar, biopolymer, and briquettes (DE CONTI et al., 2021; FOONG et al., 2020; GLANPRACHA; ANNACHHATRE, 2016; HASAN et al., 2015; LIN et al., 2021; LUCHESE; RODRIGUES; TESSARO, 2021; MADEIRA et al., 2017b; MURATA et al., 2021; SIVAMANI et al., 2018; VIRUNANON et al., 2013). This approach follows the circular bioeconomy and new policies on using waste for energy production (MPOFU; OYEKOLA; WELZ, 2021; SODHI et al., 2022). CSW and CWW have been studied due to their intrinsic nutrient properties (MOURA et al., 2018). However, there are toxic substances that must be removed for further application. On the other hand, studies using CB to obtain VAP is still scarce, showing a gap that can contribute to the circular economy (DÍAZ et al., 2020). Cassava waste obtains sustainable alcohol and acceptable chemical compounds (OGHENEJOBOH et al., 2021).

The CWW can cause environmental damage due to the high concentrations of BOD, COD, and CN (OGHENEJOBOH, 2015). In addition, CN and its derivatives cause several environmental and human health problems (SHARMA; AKHTER; CHATTERJEE, 2019). Due to CN toxicity, implementing cost-effective methods for CN removal is crucial (FENG et al., 2022). Hence, the need to remove CN from cassava wastes is essential to avoid environmental contamination, and due to their composition, VAP can be obtained concomitantly.

2.3 CYANIDE DEGRADATION IN CASSAVA WASTE

Over the years, physicochemical and biotechnological technologies have been used and improved to remove dangerous contaminants such as CN (VEDULA; DALAL; MAJUMDER, 2013). Besides the cassava industry, other processes, such as pesticides, cosmetics, electroplating, mining activities, galvanizing, and jewelry, contribute to the increase of CN in the environment (GUPTA; SREEKRISHNAN; SHAIKH, 2018; KJELDSEN, 1999; KUYUCAK; AKCIL, 2013; LUQUE-ALMAGRO; MORENO-VIVIÁN; ROLDÁN, 2016). In addition, anthropogenic forms of CN^- and HCN can enter the environment in several ways, being deposited or leached into the soil and even into water bodies (ITOBA-TOMBO et al., 2017). Therefore, developing technologies that mitigate or reduce these substances before they reach the environment is crucial.

Physicochemical removal processes tend to be effective only with free CN and weak metal CN complexes, but since CN strongly bounds to metals, the process is impaired. In addition, it could be expensive, complex to operate, and generate more toxic by-products (ADEMAKINWA; AGUNBIADE; FAGBOHUN, 2021; ALVILLO-RIVERA et al., 2021; AN et al., 2018).

The biological removal of pollutants has intensified over the past decade, mainly through anaerobic digestion (AD) due to waste remediation, energy production, and nutrient recovery (KARKI et al., 2021; VEDULA; DALAL; MAJUMDER, 2013). Under anaerobic and aerobic conditions, CN complexes degrade mainly free CN (GUPTA; SREEKRISHNAN; SHAIKH, 2018; KJELDSEN, 1999). Both processes have been used to remove CN present in effluents from several sources (GUPTA; SREEKRISHNAN; SHAIKH, 2018; KAEWKANNETRA; CHIWES; CHIU, 2011; PAIXÃO et al., 2000; POTIVICHAYANON et al., 2020). In this context, biological processes efficiently remove CN, including cassava waste. The aerobic and anaerobic treatments break and transform the CN into simple non-toxic substances (GUPTA; AHAMMAD; SREEKRISHNAN, 2016; GUPTA; SREEKRISHNAN; SHAIKH, 2018).

2.3.1 Biological CN degradation pathways

In the biological processes, CN-degrading microorganisms produce enzymes capable of metabolizing CN, providing degradation or transformation products that reduce toxicity, or even using CN as a substrate (DUBEY; HOLMES, 1995; GUPTA; BALOMAJUMDER; AGARWAL, 2010; PARK; TREVOR SEWELL; BENEDIK, 2017). Microorganisms (mainly bacteria and fungi) convert free CN and metal complexes into bicarbonate and NH_3, while free metals are adsorbed within the biofilm or precipitated out of the solution (DASH; GAUR; BALOMAJUMDER, 2009). The CN degradation pathway (**Table 03**) is well known, as well as the enzymes involved in the CN species degradation or conversion to less harmful byproducts (MEKUTO; NTWAMPE; AKCIL, 2016). Five CN degradation biotic pathways have been reported in the literature for biological processes: i) hydrolytic, ii) oxidative, iii) reductive, iv)substitution/transfer process and v) synthases (CABELLO et al., 2018; GUPTA; BALOMAJUMDER; AGARWAL, 2010; MALMIR et al., 2021; NOVAK et al., 2013; SHARMA; AKHTER; CHATTERJEE, 2019; VEDULA; DALAL; MAJUMDER, 2013).

Enzymes in several microorganisms convert CN using it as a source of carbon and nitrogen (GUPTA; BALOMAJUMDER; AGARWAL, 2010; RAYBUCK, 1992). Fungal species utilize CN hydratase for CN conversion (VEDULA; DALAL; MAJUMDER, 2013). As presented in **Table 03**, ammonia is obtained at the end of many pathways, and some have intermediate formation during CN conversion (e.g., CH_4, $HCONH_2$). Kandasamy et al. (2015) reported that the CN and glucose concentration were directly related to CN removal, and at the end of the process, metabolic products were formed (e.g., ammonia and formate), indicating a hydrolytic pathway.

Table 03 – Cyanide (CN) degradation mechanism for each pathway.

Reactions Pathway		Mechanism	Suggested biological process
Hydrolytic	Cyanide hydratase (CHT)	$HCN + H_2O \rightarrow HCONH_2 \rightarrow HCOOH + NH_3$	Anaerobic
	Nitrile hydratase	$RCN + H_2O \rightarrow RCONH_2$	
	Amidase	$RCONH_2 \rightarrow RCOOH + NH_3$	
	Cyanide dehydratase (CynD)	$HCN + 2H_2O \rightarrow HCOOH + NH_3$	
	Nitrilase	$RCN + H_2O \rightarrow RCOOH + NH_3$	
	Thiocyanate hydrolase	$SCN + 2H_2O \rightarrow S{=}C{=}O + NH_3 + OH^-$	
Oxidative	Cyanide monooxygenase	$HCN^- + O_2 + 2H^+ + 2e^- \rightarrow OCN^- + H_2O$	Aerobic
	Cyanide dioxygenase	$HCN + O_2 + 2H^+ + NADPH \rightarrow NADH + CO_2 + NH_3$	
	Cyanase	$HCN + NADPH \rightarrow OCN^+ + HCO^-_3 + 2H^+ \rightarrow CO_2 + NH_3$	
Reductive	Nitrogenase	$HCN + 6H + 6e^- \rightarrow CH_4 + NH_3$	Anaerobic or aerobic
Substitution/transfer	3−Cyanoalanine synthase	$HCN + Cystene \rightarrow H_2S + NC\text{-}CH_2(NH_2)COOH$	Anaerobic or aerobic
	Rhodanese	$CN^- + S_2O_3 \rightarrow SCN^- + SO_3^{2-}$	
Synthases	β-Cyanoalanine	$HCN + Cystene \rightarrow H_2S + NC\text{-}CH_2CH(NH_2)COOH$	Anaerobic or aerobic
	γ-cyano-α-aminobutyric acid	$CH_3COOCH_2CH(NH_2)CO_2H + CN^- \rightarrow NCCH_2CH(NH_2)CO_2H + CH_3COO^-$	

Source: (CABELLO et al., 2018; EBBS, 2004; FALLON, 1992; GUPTA; BALOMAJUMDER; AGARWAL, 2010; SHARMA; AKHTER; CHATTERJEE, 2019; TERADA et al., 2022; VEDULA; DALAL; MAJUMDER, 2013)

Many microorganisms use the hydrolytic pathway when CN is present in the water or soil, such as *Bacillus sp*., due to high CN-degrading activity (TERADA et al., 2022). About forty microorganisms have been reported to produce nitrilase, more than sixty nitrile hydratases, and around a hundred showed amidase activity (BHALLA et al., 2018). Therefore, as CN-degrading nitrilases (i.e., CynD and CHT) do not need secondary substrates or cofactors, their application (or microorganisms that produce them) is feasible for industrial wastewater (PARK; TREVOR SEWELL; BENEDIK, 2017). Although biological processes are well-described for CN removal, inhibition must be evaluated, and acclimatization must be followed.

Reductive pathways require nitrogenase enzymes for CH_4 production and release ammonia as an end product (GUPTA; SREEKRISHNAN; SHAIKH, 2018). Anaerobic conditions are necessary for reductive pathways (PARK; TREVOR SEWELL; BENEDIK, 2017). In the substitution pathway, thiocyanate is assimilated as an alternative nitrogen source during microbial growth (SHARMA; AKHTER; CHATTERJEE, 2019). In the substitution/transfer pathway, CN can be degraded mainly by rhodanase, but other extracellular enzymes are also secreted and might be involved in the degradation (GUPTA; BALOMAJUMDER; AGARWAL, 2010). Meanwhile, the microorganisms *B. megaterium*, *E. coli*, and *C. violaceum* can convert the *β*-cyanoalanine to asparagine and aspartate through the hydrolytic pathway (GUPTA; BALOMAJUMDER; AGARWAL, 2010; RAYBUCK, 1992). In the last decade, several papers about CN removal contained in cassava waste have been reported in the literature (**Table 04**). It is important to highlight that some studies have reported CN removal and concomitantly reusing these residues to obtain by-products, such as biogas and volatile fatty acids (VFA) (GLANPRACHA et al., 2018; GLANPRACHA; ANNACHHATRE, 2016; HASAN et al., 2015)

Table 04 - Literature reports about biological cyanide (CN) treatment

(to be continued)

Biological treatment pathway	Characteristics of Cassava Waste				Micro-organisms involved	Working conditions					Removal Efficiency (%)			Reference
	pH	COD ($mg \cdot L^{-1}$)	BOD ($mg \cdot L^{-1}$)	CN ($mg \cdot L^{-1}$)		Workload (L)	pH	Agitation (rpm)	T (°C)	DO	CN	BOD	COD	
Aerobic	5.5	16,000	NR	86	*A. vinelandii* TISTR 1094	20	7-8.5	NR	30	2 $mg \cdot L^{-1}$	90	NR	74.5	(KAEWKANNETRA et al., 2009)
Aerobic and Anaerobic	5.5 ± 0.2	16,000 ± 968	NR	86 ± 2	Mixed culture-activated sludge	30	NR	NR	30	NR	~70	NR	86	(KAEWKANNETRA; CHIWES; CHIU, 2011)
Aerobic	5.6	16,000	11,675	85.9	*A. vinelandii*	3	NR	200	30	2 $L \cdot min^{-1}$	69.7	NR	NR	(KOKSUNAN et al., 2013)
AcoD (CWW and swine manure treatment)	4.0	8,865	11.32	1.9	Anaerobic digester from swine manure	2	7.0	NR	30	NR	12.9	NR	21.6	(HASAN et al., 2015)
AcoD	4.0	NR	NR	9.8 ± 2 $mg \cdot Kg^{-1}$	CP and PM	7	7.5-7.7	50 for 10 min every 2 hour	31.1 ± 0.7	NR	92	NR	NR	(GLANPRACHA; ANNACHHATRE, 2016)
AcoD	NR	NR	NR	1.5-10	Mixed culture anaerobic sludge	0.02	7.2-7.5	NR	31 ± 1	NR	80-85	NR	NR	(GLANPRACHA et al., 2018)
ET	NR	NR	NR	534	Enzymes mixture from *T. reesei*	~0.005	5	NR	55	NR	82	NR	NR	(LATIF et al., 2019)
AD and Aerobic	4.9 ± 0.1	1,006.7 ± 10.61	532.3 ± 1.41	1.6 ± 0.23	Provinient from PSWW	Chamber 1: Sedimentation	4.9	NR	27-30	NA	43.75	26.73	23.18	(LAWAL et al., 2019)
						Chamber 2: biofilter of PKS	4.6			NA	55.56	44.28	41.59	
						Chamber 3: aeration	4.5			NR	25	33.27	32.10	
						Chamber 4: Sedimentation				NA	33.33	20.90	22.30	

Table 04 - Literature reports about biological cyanide (CN) treatment

(Conclusion)

Biological treatment pathway	Characteristics of Cassava Waste				Micro-organisms involved	Working conditions					Removal Efficiency (%)			Reference
	pH	COD ($mg \cdot L^{-1}$)	BOD ($mg \cdot L^{-1}$)	CN ($mg \cdot L^{-1}$)		Workload (L)	pH	Agitation (rpm)	T (°C)	DO	CN	BOD	COD	
Aerobic	NR	NR	NR	39.80	*S. cerevisiae*	0.065-0.165	NR	NR	30	NR	97.96	NR	NR	(HAWASHI et al., 2019)
Aerobic	4.67 ± 0.03	70,670 ± 60	5,040 ± 80	4.46 ± 0.02	*Candida tropicalis* ASY2	NR	6	150	30	NR	78.94	92.11	83.52	(THANGAVELU et al., 2020)
Aerobic	4.3 – 5.1	16,266.67 – 26,666	NR	132.92 - 252.66	*Agrobacterium tumefaciens* SUTS 1 and *Pseudomonas monteilii* SUTS 2	30	NR	NR	NR	0.35-1.5 $mg \cdot L^{-1}$	95.45	NR	78	(POTIVICHAYANON et al., 2020)
Aerobic	NR	NR	NR	20.87	*S. cerevisiae*	NR	NR	NR	room	NR	55.86 – 56.06	NR	NR	(HIDAYAT et al., 2020)
ET	5.0 ± 1.0	10,000 ± 763	NR	102 ± 12	Rhodanese of *Aureobasidium pullulans* free and immobilized	~0.006	4.5-11.0	150	20-30	NR	55-74	NR	NR	(ADEMAKINWA; AGUNBIADE; FAGBOHUN, 2021)

Footnote - rpm: rotations per minute; NR: not reported; COD: chemical oxygen demand; BOD: biochemical oxygen demand; HRT: hydraulic residence time; T: temperature; CWW: cassava wastewater; CP: cassava pulp; PM: pig manure; SSS-CSTR: single stage semi-continuous stirred tank reactor; SBR: sequencing batch reactor; F-SBR: fixed-film sequencing batch reactor; MFC: microbial fuel cell; VFCW: vertical flow constructed wetland; CSTR: continuous stirred tank reactor; PSWW: Poultry slaughterhouse wastewater; PKS: palm kernel (Elaeis guineensis) shell; DO: dissolved oxygen; ET: enzymatic treatment; AcoD: anaerobic co-digestion; AD: anaerobic digestion; NA: not applicaded.

2.3.2 Aerobic process

The CN degradation by aerobic processes is related to some groups of microorganisms, mainly to the genera *Azotobacter, Candida, Agrobacterium,* and *Pseudomonas* (**Table 04**) (KAEWKANNETRA et al., 2009; KJELDSEN, 1999; KOKSUNAN et al., 2013; POTIVICHAYANON et al., 2020; THANGAVELU et al., 2020). The *A. vinelandii* TISTR 1094 has been reported as a strain involved in CN reduction by the nitrogenase, and the CN degradation results in CH_4 and NH_3 production (KAEWKANNETRA et al., 2009). Corroborating, Koksunan et al. (2013) have confirmed that the enzyme nitrogenase is responsible for CN degradation and methane production. Although the genus *Azotobacter* is an aerobic microorganism, the authors observed that when ammonia is limited in CWW, CH_4 production occurs due to nitrogenase enzyme activity. However, the microorganism can use the substitution pathway due to the enzyme rhodanese (SHARMA; AKHTER; CHATTERJEE, 2019). The CN reduction mechanism could be associated with the *A. vinelandii* N_2-fixing enzymes (KAEWKANNETRA et al., 2009). CN degradation rate is higher in the exponential growth phase, suggesting that CN removal is linked to *A. vinelandii* metabolic activity, indicating the use of CN as an alternative nitrogen source; a similar result was also found in *K. oxyotoca* (KAEWKANNETRA et al., 2009; KAO et al., 2003). Nonetheless, Koksunan et al. (2013) also used *A. vinelandii* and found that after 60 hours, in the stationary phase the CN concentration was reduced by 75.7 % and in the mid- exponential phase it was 55.7 %. Furthermore, to achieve higher CN removal, it might be necessary to perform the microorganisms acclimatization or the enrichment of a mixed culture with known CN degrader microorganisms. The CN removal in the range of (initial concentration of 86 ± 2 $mg \cdot L^{-1}$) 70 to 90% was reached by the bacteria due to its acclimatization mechanism using activated sludge with CWW, which was gradually replaced by synthetic wastewater containing 6-14 $mgCN \cdot L^{-1}$ (KAEWKANNETRA; CHIWES; CHIU, 2011).

Thangavelu et al. (2020) used *Candida tropicalis* ASY2 to remove CN from cassava wastewater and concomitantly produce lipids. The wastewater, rich in starch, allowed the lipid yield production of 48.56%, reaching 78.94% CN removal without any nutritional supplementation. Furthermore, the CN degradation by *C. tropicalis* ASY2 was attributed to the presence of the enzymes such as CN hydratase, nitrile hydratase, thiocyanate hydrolase, nitrilase, and cyanidase. Potivichayanon et al. (2020) reported that in a mixed culture of *Agrobacterium tumefaciens* SUTS 1 and *Pseudomonas monteilii* SUTS 2, the decrease in CN

concentration is related to an increase in the synthesis of by-products such as NH_3-N, NO_2^- and NO_3^-. Besides, the HRT of 5 days achieved higher CN removal (95%) than 7 (89.21%), suggesting that acclimatization played an important role. In addition, the authors estimated the cost of 0.11 US$·m^{-3} to remove the CN in the initial concentration of 208.93 mg·L^{-1} from the CWW.

The treatment using sequential anaerobic and aerobic reactors (five chambers) resulted in the final CN removal of 73.56 % (LAWAL et al., 2019). Chamber 3 was working out with optimized aerobic conditions (aeration of 2.8 L·min^{-1}), but had the lowest CN removal efficiency (25%), probably due to the low CN-converting microorganisms growth, although the aeration rate was in an optimized condition (LAWAL et al., 2019). The acidic pH and low CN (i.e., 1.6 mg·L^{-1}) levels found in CWW were most probably due to the wastewater collected in the industry fermentation tank, so the acidification during the process is evident, leading to CN volatilization. In addition, the pH is still consistent with others found in the literature.

Aerobic processes are applied in different industries to remove recalcitrant contaminants and organic load. Some configurations can be adopted, such as a three-stage bioreactor to remove phenol, CN, ammonia, and organic load (MANEESH et al., 2018). The results achieved for CN removal surpassing 99% when the flow rate was 1 m^3·day^{-1} (MANEESH et al., 2018). Furthermore, this configuration also removes high concentrations of organic matter (90%). Corroborating that aerobic biologic processes are an excellent alternative for CN removal in mining and steel industries (GUAMÁN GUADALIMA; NIETO MONTEROS, 2018; LUQUE-ALMAGRO; MORENO-VIVIÁN; ROLDÁN, 2016).

Prospecting and isolating microorganisms in CN-contaminated sites may contribute to discovering new CN degraders. *Pseudomonas stutzeri* and *Bacillus subtilis* isolated from soil next to a cassava industry were able to remove 72.0% and 66.9% of CN, respectively, in the contaminated soil sample. The mixed culture degraded 88.5% of the CN content after 10 days with a concentration of 0.218 mg·CN·g^{-1} in the soil (NWOKORO; DIBUA, 2014). Therefore, evaluating which microorganisms are responsible for CN degradation is crucial for understanding the process. As expected, the complexity of the carbon source will also affect the CN removal. For example to *Pseudomonas*, when the carbon source is lactate, the bacteria can remove up to 100 mg·CN·L^{-1}, unlike when phenol is the carbon source (RAYBUCK, 1992; WHITE et al., 1988). Probably due to the less toxic effect of CN with lactate as a carbon source than CN with phenol (GAUDY et al., 1988). Also, at the concentration of 24 mgCN·L^{-1}, the

CN degradation was complete due to the use of potassium lactate as substrate (KARAVAIKO et al., 2000).

Several studies used *Saccharomyces cerevisiae* (see **Table 04**), a non-pathogenic and facultative anaerobic yeast, for CN detoxification from many wastes, including cassava waste (HAWASHI et al., 2019; HIDAYAT et al., 2020; MURATA et al., 2021; OBOH, 2003; SHEN; WU; XU, 2021). A solid-state fermentation using *S. cerevisiae* allowed an efficient CN degradation (97.96 %) in CL with an initial CN concentration of 39.80 $mg \cdot L^{-1}$ (HAWASHI et al., 2019). Using pure culture of *S. cerevisiae* and *tape* yeast (a consortium of yeast, fungal and bacterial) in CP, with fermentation periods of 0, 24, 48, 72, 96, and 120 hours, it was observed that the pure culture could grow better than the strange culture, and CN degradation of 55.86% (HIDAYAT et al., 2020). Two new bacteria designated as BN1 (identified as *Halomonas*) and DNB (candidate for a novel taxon), capable of tolerating up to 350 $mg \cdot L^{-1}$ of CN at pH 9.5 and temperature 25 °C, showed CN reduction of 66 and 50 %, respectively (KHAMAR; MAKHDOUMI-KAKHKI; MAHMUDY GHARAIE, 2015). Moreover, when using a medium containing glucose as a carbon source, these isolates tolerate up to 1,200 $mg \cdot L^{-1}$ of CN.

2.3.3 Anaerobic process

Anaerobic digestion (AD) has gained attention mostly due to producing biogas as a product of organic matter degradation and being a compact and efficient process. Besides removing CN, AD has also other advantages over aerobic degradation, such as low sludge volume, and low energy demand (GUPTA; SREEKRISHNAN; SHAIKH, 2018; LUQUE-ALMAGRO et al., 2018; NOVAK et al., 2013). High-rate anaerobic reactors, especially the established Upflow Anaerobic Sludge Blanket (UASB), can be applied in different industries, with numerous advantages, such as high organic matter removal efficiency, simple construction, and low chemical consumption (STAZI; TOMEI, 2021). Moreover, many researchers have found that CWW are suitable for the AD (**Table 04**), especially using UASB reactor (JIJAI et al., 2015). Gijzen et al., 2000 tested a laboratory-scale UASB reactor for the CN biodegradation in synthetic effluent with concentrations of 0-125 $mgCN \cdot L^{-1}$, reaching a removal efficiency of 91-93 %. However, there has been inhibition in the activity of acetolactic and hydrogenotrophic methanogens. Another study fed a UASB reactor with synthetic effluent (0-42 $mgCN \cdot L^{-1}$) for 342 days, followed by feeding with CWW (0- 10 $mgCN \cdot L^{-1}$) for 59 days and obtained 93-98 % of CN removal (Annachhatre and Amornkaew, 2001). Likewise, after

the acclimatization period, the synthetic wastewater was gradually replaced by CWW, obtaining CN removal up to 97 % (Oliveira et al., 2001a; Oliveira et al., 2001b). Efficient CN removal can be achieved due to the inoculum acclimatization and the existence of microorganisms that can assimilate and use CN as a nitrogen and carbon source (DOBLE; KUMAR, 2005; DUBEY; HOLMES, 1995; LUQUE-ALMAGRO; MORENO-VIVIÁN; ROLDÁN, 2016; OJAGHI et al., 2018; RAYBUCK, 1992).

The nutritional C/N ratio imbalance in CWW limits the biological treatment (KAEWKANNETRA et al., 2009). Glanpracha and Annachhatre (2016) performed the anaerobic co-digestion of CP and pig manure (PM) and reported the C/N ratio of 35:1 as the ideal condition for the processes. Using reactors operating in batch mode, the co-digestion of PM and CP resulted in CN removal efficiency of 80-85 % after 3 days of acclimatization (GLANPRACHA et al., 2018). However, in the anaerobic co-digestion operating in semi-continuous mode, the microbial community was fully acclimatized after 110 days, allowing CN removal of 92 %. Moreover, the inhibitory effects CN (with an initial concentration of 9.8 ± 2 mgCN·Kg^{-1}) were avoided (GLANPRACHA; ANNACHHATRE, 2016). Furthermore, due to the pH 4.0 of CWW, CN was mostly available in the form of cyanohydrins. Moreover, after 110 of acclimatization the microbial community was adapted and removing CN. In addition, the CN biodegradation pathway was hydrolytic, confirmed by formate and NH_4^+ formation.

Temperature and pH are essential parameters involved in the efficiency of CN degradation (GUPTA; BALOMAJUMDER; AGARWAL, 2010). Therefore, these must be evaluated and optimized for each waste generated in the cassava industry due to the diversity in the physic-chemical composition. In this perspective, Hasan et al. (2015) obtained a higher yield of VFA (3.4 g·L^{-1}) at 30 °C and 3 g·L^{-1} of $NaHCO_3$ using an acidogenic reactor fed with CWW. In contrast to the increase in VFA production, CN and COD removal was low (12.9% and 21.6%, respectively), suggesting that the biodegradation process was impaired due to CN inhibitory effect. Other factors, such as the acclimatization of the inoculum with CN, may have contributed to the low pollutants removal.

Anaerobic granular sludge from a UASB reactor seems to have the microbial community necessary to degrade CN. Furthermore, the granular sludge is a structured aggregate that also helps to promote its degradation (ANDRADE et al., 2020; COLIN et al., 2007; GIJZEN; BERNAL; FERRER, 2000; GUPTA; AHAMMAD; SREEKRISHNAN, 2016; GUPTA; SREEKRISHNAN; SHAIKH, 2018; NOVAK et al., 2013). The anaerobic

community in granular sludge could remove CN from synthetic effluent under a long operation time and reach a high threshold so that the process does not inhibit (GUPTA; SREEKRISHNAN; SHAIKH, 2018). The CN degradation might be linked to the phylum Firmicutes and the archaeal genus *Methanosarcina* sp. present in granular sludge (from a reactor treating brewery wastewater) (NOVAK et al., 2013). Granular anaerobic sludge is also used to treat starch-rich wastewater from the food industry (QIN et al., 2018; SUN et al., 2012; WEIDE et al., 2019). These studies suggest that using anaerobic granular biomass may be promising to biodegrade CN and treat starchy wastes such as CWW.

Studies show that using wastes such as palm kernel and coconut husk can remove toxic pollutants from industrial wastewater and be eco-friendly (ISA et al., 2022; PACKIALAKSHMI et al., 2021). In another research evaluating anaerobic biofiltration chamber in an SBR, composed by four chambers, was filled with palm kernel husk (PKH) and poultry slaughterhouse sludge achieved the removal efficiency to HCN, COD and BOD of 55.56 %, 41.59 % and 44.28 %, respectively (LAWAL et al., 2019). Due to higher CN removal compared to the other chambers in the study, authors suggest a high presence of unicellular anaerobic CN-degrading microorganisms in the biofiltration chamber. Furthermore, they suggest that a higher HRT could bring the CN content to more acceptable levels. However, the CN adsorption on PKH was not evaluated, which may overestimate the removal efficiency. PKH is a cheap alternative, as the authors point out, but other materials can be used for CN degradation.

The UASB granules from the brewery show the potential for CN removal, probably due to the aggregate structure, which is an essential feature for CN degradation (NOVAK et al., 2013). Microorganisms found in this waste, such as *S. cerevisiae*, that are discarded in brewery wastewater can provide economic gains due to reuse to obtain by-products and CN removal (RUBIO et al., 2020). Dehghani et al. (2016) performed *in vitro* experiments for CN degradation. The authors also optimized pH and temperature conditions and identified that pH 8.0 and 30 °C had greater efficiency, obtaining the removal of 58.42%, corroborating with (HASAN et al., 2015). Again, this yeast can degrade CN, showing an economically attractive solution because breweries release this yeast into wastewater (RUBIO et al., 2020). Furthermore, *S. cerevisiae* can also biodegrade CN by the nitrilase pathway presented in **Table 03** (SHEN; WU; XU, 2021). In addition, the yeast can concentrate enormous quantities of by-products, such as thiamin, nicotinic, and biotin (ASOGWA; OKOYE; ONI, 2017). It is evident

that many studies address the CN degradation using *S. cerevisiae* due to satisfactory results, but other microorganisms are studied and deserve to be highlighted.

Numerous studies using cassava processed waste are performed with pure culture or mixed microorganisms in different biological processes (**Table 04**). The use of mixed culture has an advantage due to the interactions between these microorganisms, which can promote the generation of products of interest. There is an increase of studies analyzing AD in single or two steps with CWW to produce biogas, H_2, and VFA (CREMONEZ et al., 2021). However, many two-step studies need to analyze the removal and inhibition effect of CN in obtaining VAP.

2.4 THE CN INFLUENCES THE DEGRADATION OF OTHER POLLUTANTS AND VAP GENERATION

There needs to be more understanding of how CN can interfere in the cassava treatment and obtaining by-products from these residues. Many studies have not analyzed this interferent, but understanding how CN can be harmful or beneficial to treatment and microorganisms must be addressed (ARAUJO et al., 2021; SUN et al., 2012; THANGAVELU et al., 2021; ZHANG et al., 2010). However, the number of CN-degrading microorganisms is considerable; the insight for this analysis in cassava waste is only sometimes studied because, in some studies, the pretreatment removes CN. According to Dash et al., (2009), the sensitivity of CN-degrading microorganisms must be observed, as their growth is impaired when it exceeds the limits, resulting in a low degradation of CN and organic matter. Moreover, the CN degradation efficiency may be directly proportional to the COD removal, suggesting an interlinked with CN levels (KAEWKANNETRA et al., 2009).

Microbial degradation involves enzymatic pathways and specific conditions, such as adequate pH, temperature, and adaptation to the CN concentration (GUPTA; BALOMAJUMDER; AGARWAL, 2010). For example, at 35 °C *Streptomyces tritici* D5 can tolerate and degrade 100 mM concentration of KCN (ANUPONG et al., 2022). The leaching and degradation processes of iron-complex CN are associated with redox and pH conditions (KJELDSEN, 1999). Several studies performed CN degradation through AD achieving good results. However, some microorganisms, such as *archaea* methanogens, are more sensitive to CN inhibition than bacteria (FEDORAK; ROBERTS; HRUDEY, 1986). Some hydrogenotrophic methanogenic groups can grow and produce CH_4 in CN-acclimatized sludge (GUPTA; AHAMMAD; SREEKRISHNAN, 2016). Acetoclastic methanogens are more

affected by CN than by hydrogenotrophic methanogens (GIJZEN; BERNAL; FERRER, 2000; GUPTA; AHAMMAD; SREEKRISHNAN, 2016). Even though the inhibitory effects of CN on methanogens this is reversible or might be avoided by proper adaptation (ANNACHHATRE; AMORNKAEW, 2001; DASH; GAUR; BALOMAJUMDER, 2009; GIJZEN; BERNAL; FERRER, 2000; GLANPRACHA et al., 2018; GUPTA; AHAMMAD; SREEKRISHNAN, 2016; PAIXÃO et al., 2000).

Gupta et al. (2016) used increasing CN concentrations (10-55 $mg \cdot L^{-1}$) to describe the acclimatization and inhibitory effects in the microbial community. The authors showed a lower CH_4 production (i.e., 35 and 19 %) on the 35^{th}, and 86^{th} day, respectively, when the CN concentration was 10 and 55 $mg \cdot L^{-1}$, respectively. In the study, the authors demonstrated that the VFA production rate was slower, and production of acetate (>1500 $mg \cdot L^{-1}$) was stimulated. In addition, it was reported that acetate accumulation was possibly impaired due to inhibitory effect of the methanogens by the CN. The authors highlighted that there was a predominance of *Methanobacteriales* and *Clostridiales* in the presence of CN which may be linked to their tolerance. According to Novak et al. (2013), the CH_4 yield decreased as the CN concentration increased, and at 85 days, the yield increased, suggesting the adaptation of microorganisms.

Therefore, reactor acclimatization with increasing gradual CN concentrations is recommended to attenuate the inhibition of microorganisms (ANNACHHATRE; AMORNKAEW, 2001; GIJZEN; BERNAL; FERRER, 2000). This suggestion favors the CN removal and other pollutants, helping to obtain products of interest, promoting the growth of specific microorganisms, and TRH. To avoid possible inhibitory effects of CN, biochar or hydrochar can be used to promote protection for methanogenic archaea, acidogenic and acetogenic bacteria (CAVALI et al., 2022).

The *K. oxytoca* is able to tolerate and biodegrade CN in addition to producing CH_4 and NH_3 (KAO et al., 2003). The authors report that despite the gradual acclimatization with CN, the formation of NH_3 as a by-product might contribute to the inhibitory effect. Result demonstrates that it is not only CN that can cause an inhibitory effect, so NH_3 reduction or conversion strategies should be adopted. The direct effects of CN complexes on microorganisms are also studied in ethanol production by *P. kudriavzevii* LC375240 and *S. cerevisiae* LC269108 from CP and CPe (MURATA et al., 2021). The inhibition was confirmed by measuring the intracellular pyruvate biosynthesis in the yeast cells exposed to CN, indicating that CN interferes with the metabolic pathway for ethanol production. Thus, using known CN-degrading bacteria has shown to be an interesting approach to promote a higher biodegradation

rate, in addition to forming by-products, suggesting pathways of CN degradation (RAZANAMAHANDRY et al., 2019).

Notably, CN may affect some groups of microorganisms in AD, resulting in the by-product accumulation (e.g., acetate) that contributes to its inhibitory effect. Therefore, the acetoclastic methanogens are more sensitive microorganisms, affecting CH_4 production more than VFA and H_2 production. Although the influence of CN on the processes using synthetic effluent was already reported, no studies in the literature observe the interference of CN to obtain VAP using cassava waste (e.g., CP, CPe, CWW). However, as CN causes less effects on hydrogenotrophic methanogenic, conditions to maximize the productive yield of biohydrogen must be adopted.

2.5 TRENDS IN CYANIDE REMOVAL FROM CASSAVA WASTE

Conventional physicochemical methods are ineffective in removing high concentrations of CN in industrial wastewaters; in addition, they present other disadvantages such as high costs, not being eco-friendly, maintenance difficulty, and production of toxic compounds (ESKANDARI et al., 2019; GUAMÁN GUADALIMA; NIETO MONTEROS, 2018; GUPTA; SREEKRISHNAN; SHAIKH, 2018; KOKSUNAN et al., 2013; KUMAR et al., 2017; MEKUTO; NTWAMPE; AKCIL, 2016). On the other hand, CN removal from industrial wastewater can be carried out by microorganisms that can degrade it (LUQUE-ALMAGRO; MORENO-VIVIÁN; ROLDÁN, 2016). Therefore, the biological models are an interesting approach once CN can be used as a carbon and nitrogen source, generating VAP, such as CH_4, VFA, and H_2, thus promoting green chemistry.

Using UASB granular inoculum from industries such as of brewery in future studies may play a crucial role in including the circular economy in the cassava industry sector. Furthermore, isolation and identification of other microorganisms capable of biodegrading CN into non-hazardous substances, such as *S. cerevisiae* are also necessary (RUBIO et al., 2020; SHEN; WU; XU, 2021). The use of inoculum rich in different microorganisms can improve the CN biodegradation process, for example, using biofilm-forming bacteria can promote the immobilization of nitrile-degrading bacteria and increase the removal of CN (AN et al., 2018). The CN inhibitory effects are well studied on methanogenic microorganisms, therefore, there is a gap in analyzing these effects on acidogenic and acetogenic bacteria in obtaining VAP. Changes in the process parameters can provide a higher CN removal efficiency. For example,

the increase of the HRT and changes in pH and temperature (AN et al., 2018; HUERTAS et al., 2010; KHAMAR; MAKHDOUMI-KAKHKI; MAHMUDY GHARAIE, 2015; LUQUE-ALMAGRO et al., 2005; SHARMA; PHILIP, 2015). Notably, acclimatization is the key to CN degradation from cassava waste and producing products of interest. CN biodegradation using *P. putida* and *P. stutzeri*, isolated from the coke-oven, achieved the CN removal of 80.6 % (SINGH; ARORA; SACHAN, 2018). The strains could degrade and tolerate high CN concentration (340 $mg \cdot L^{-1}$). In addition, BOD and COD reduced by more than 50 %, showing a good alternative for biodegradation in CWW. The ability to tolerate and degrade high concentrations of CN is probably due to the lack of influence on the CN dihydrate present in CN concentration that has not influenced the CN dihydrates present in *P. stutzeri* (GUPTA; BALOMAJUMDER; AGARWAL, 2010).

Electro-biodegradation using *B. pumilus* experiments has shown that by adding electrons, the process reaction time decreases, and CN degradation efficiency increases significantly (OJAGHI et al., 2018). The efficient CN removal using electro-biodegradation was 98.6, 88.27, and 99.7 % in 36, 72, and 137 hours of the operation time, respectively, against 98.5, 98.8, and 65.3 % in 230, 396, and 622 hours in biodegradation, respectively. Moreover, the authors highlight the importance and efficiency of the electro-biodegradation of CN in aqueous solutions. In a combined biological system of the anaerobic, anoxic, and aerobic reactor, in HRT of 6 days, the anaerobic condition removed more than 75% of CN, the efficiency being almost unchanged when the CN concentration increased from 27 to 68 $mg \cdot L^{-1}$ (CHAKRABORTY; VEERAMANI, 2006). The results obtained from the studies described above corroborate that adopting biological models to remove CN is viable, efficient, and products of interest can be obtained. MFCs have gained prominence in the last decade and are used in the treatment of CWW. Quan et al. (2014) evaluated the pre-fermentation of the CWW before entering the MFC and the aeration of the anodic chamber. The removal efficiency of TCOD was 62.5 ± 3.5 % when applied to pre-fermentation, increasing energy generation when compared to without pre-treatment. The power density jumped from 159.9 to 437.1 ± 15.6 $mW \cdot m^{-2}$.

2.6 CONCLUSIONS

Cassava industry generates significant amounts of liquid and solid effluents with their composition varying according to the process, especially for CN. The methods for determining CN in CWW are diverse, and there is a need to standardize the methodology. Anaerobic and aerobic biological processes are efficient in removing CN from these wastes. Although biological processes remove CN, adaptation proves to be a crucial role in the process, promoting the growth of microorganisms capable of degrading CN in higher concentrations. Using anaerobic granular sludge in AD seems to increase the tolerance to CN and helps to degrade it. The number of cataloged microorganisms capable of converting CN from CWW into less toxic compounds is high, but it is also necessary to identify other microorganisms. Several processes show the potential to remove CN from CWW, such as electro-biodegradation, two-step AD, and rotating biological contactors. CN is an inhibitory compound in biological processes, but strategies such as acclimatization, increase in HRT, promote the microbial diversity in the process can be adopted to minimize these damages.

Chapter

3

Experimental article: Cyanide removal present in cassava wastewater through acidogenic reactor and anaerobic digestion

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In this chapter, the cyanide removal in an acidogenic and complete anaerobic digestion reactor was evaluated, and the relationship between the production and composition of biogas, pH, organic matter removal, nitrogenous compounds, volatile fatty acids production, and finally the relative abundance of the community microbial was established.

3 EXPERIMENTAL ARTICLE: CYANIDE REMOVAL PRESENT IN CASSAVA WASTEWATER THROUGH ACIDOGENIC REACTOR AND ANAEROBIC DIGESTION

3.1 INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is an important food source for several countries due to the social, economic and industrial importance (DEVI et al., 2022; JAMPA et al., 2022). It is the third most important source to obtain calories in the tropics, after just rice and maize (SORNYOTHA; KYU; RATANAKHANOKCHAI, 2010; ZHANG et al., 2016), as cassava can be consumed just cooked or as feedstock to produce food products such as *akyeke* (or attieke), flour, *fufu*, *garri*, lafun, kasili, sago and tapioca (ADAMS et al., 2009; AMORIM et al., 2018; FALADE; AKINGBALA, 2010; RAY; SIVAKUMAR, 2009). Moreover, it is used to produce ethanol (KHANPANUEK et al., 2022; KUBO et al., 2014).

The cassava processing industry generates a large amount of liquid waste, mainly in root washing and pressing the milled roots (FOONG et al., 2020; LUCHESE; RODRIGUES; TESSARO, 2021; OGHENEJOBOH et al., 2021; OLAOYE et al., 2020). Cassava wastewater (CWW), which contains carbohydrates and high organic concentration due to residual sugars released from cassava pressing (COUTINHO RODRIGUES et al., 2021; CREMONEZ et al., 2021; OGHENEJOBOH et al., 2021; PRASERTSUNG et al., 2019). The chemical oxygen demand (COD) in CWW is high and variable (16,666 – 92,441 $mg \cdot L^{-1}$) (COUTINHO RODRIGUES et al., 2021; POTIVICHAYANON et al., 2020). On the other hand, low pH (4.0 – 5.0) is a well-known characteristic (ADEMAKINWA; AGUNBIADE; FAGBOHUN, 2021; HASAN et al., 2015; KAEWKANNETRA; CHIWES; CHIU, 2011; QUAN et al., 2014). It is estimated that the flour industry produces from 0.28 $m^3 \cdot t^{-1}$ to 0.6 $m^3 \cdot t^{-1}$ of CWW (ARAÚJO et al., 2014; DE CARVALHO et al., 2018). In addition, cassava roots contain cyanogenic glycosides (CNG) (e.g., linamarin and lotaustralin), which undergo enzymatic hydrolysis by linamarase (*β*-glucosidase) and releases hydrocyanic acid (HCN) as the end product (TORKAMAN et al., 2021; ZHONG et al., 2020). Therefore, cassava processing releases CNGs and cyanide (CN) compounds in CWW, which must be removed to avoid damage to the environment (CHISTÉ et al., 2010; COUTINHO RODRIGUES et al., 2021; LUCHESE; RODRIGUES; TESSARO, 2021). Physical-chemical and biological processes are used for CN degradation, but the physical-chemical ones have disadvantages, such as inefficiency in

removing high concentrations of CN, effective only with free CN and weak metal CN complexes, high costs, difficult maintenance and production of toxic compounds (ALVILLO-RIVERA et al., 2021; ESKANDARI et al., 2019; GUAMÁN GUADALIMA; NIETO MONTEROS, 2018; VEDULA; DALAL; MAJUMDER, 2013). Furthermore, physical-chemical processes are not indicated to treat wastewater with a high organic load, such as CWW, due to the "transfer" of organic matter rather than "converted" or "decomposed" (KONG et al., 2019). On the other hand, the microbial community from a biological process can use CN as a nutrient source or convert it to other non-toxic by-products (GUPTA; BALOMAJUMDER; AGARWAL, 2010; GUPTA; AHAMMAD; SREEKRISHNAN, 2016; LUQUE-ALMAGRO et al., 2018; PEREIRA; ARRABAÇA; AMARAL-COLLAÇO, 1996). However, the CN biodegradation efficiency depends on the microbes presence, such as *Saccharomyces cerevisiae*, *Pseudomonas pseudoalcaligenes*, and *Pseudomonas stutzeri*, with the metabolic capacity to convert CN compounds into less hazardous products (BAXTER; CUMMINGS, 2006; CABELLO et al., 2018; DEHGHANI et al., 2016; HUERTAS et al., 2010; IBÁÑEZ et al., 2017; NWOKORO; DIBUA, 2014; SHEN; WU; XU, 2021; SINGH; ARORA; SACHAN, 2018).

Cassava industry waste can be used to obtain volatile fatty acids (VFA) and biohydrogen (H_2) in the acidogenic reactor or methane (CH_4) in a reactor performing full anerobic digestion (AD) (CREMONEZ et al., 2021; MADEIRA et al., 2017a, 2017b). In addition, AD is applied in many CN biodegradation studies (ANNACHHATRE; AMORNKAEW, 2001; FALLON et al., 1991; FETTIG et al., 2013; GLANPRACHA et al., 2018; HASAN et al., 2015; NOVAK et al., 2013; WATTHIER et al., 2019). Moreover, CN is a potential inhibitor for microorganisms, especially the methanogenic (GIJZEN; BERNAL; FERRER, 2000; GUPTA; AHAMMAD; SREEKRISHNAN, 2016). On the other hand, these studies did not evaluate the CN removal cassava wastewater in acidogenic reactors, that is, with methanogenic *archaea* inhibition. However, acclimatization for an adequate period may overcome this inhibitory effect, allowing CN removal and obtaining added-value products (GIJZEN; BERNAL; FERRER, 2000; GLANPRACHA et al., 2018; GUPTA; AHAMMAD; SREEKRISHNAN, 2016). To the best of our knowledge, this is the first study evaluating the CN removal in batch reactors with and without methanogenic inhibition (AR and CAD, respectively). In addition, the production of added-value products was also evaluated in these two configurations. Lastly, the changes in the microbial community were observed.

3.2MATERIALS AND METHODS

3.2.1 Chemicals reagents

Ninhydrin (1,2,3-indantrione monohydrate) ACS reagent, 2-bromoethanesulfonate acid sodium salt (BES) 98%, butyric, formic, valeric and propionic acid were purchased from Legacy Sigma-Aldrich. Ultrapure water was used in the below analyzes (Millipore, simplicity model). Standard CN solution (993 ± 7 mg·L^{-1}) (QMC, Florianopolis, Brazil). Sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3), sodium bicarbonate ($NaHCO_3$), sodium chloride (NaCl), potassium dichromate ($K_2Cr_2O_7$), potassium biphthalate ($C_8H_5O_4K$), mercury iodide red (HgI_2), ammonium chloride (NaH_4Cl), phenolphthalein, sulfuric acid (H_2SO_4) P.A., and sodium nitrate ($NaNO_3$) P.A. (Neon, Brazil). Mercury sulfate ($HgSO_4$), silver sulfate (Ag_2SO_4), potassium iodide (KI), phenol (C_6H_5OH), salicylic, and acetic acid P.A. (Vetec, Brazil). Sulfuric acid P.A. (Química Moderna, Brasil). Yeast extract (Kasvi, Brazil).

3.2.2 Cassava waste and inoculum characterization

The effluent from the pressing cassava (CWW) was obtained from a flour manufacturing enterprise, located in the city of Sangão, state of Santa Catarina/Brazil. After sampling, CWW was immediately frozen for transportation, then stored at −80 °C until physicochemical characterization (ABNT, 9898) or CN degradation assays. The characterizations of CWW are described in **Table 02**. Considering the application for CN degradation in AD, the inoculum used was anaerobic granular sludge from the Upflow Anaerobic Sludge Blanket (UASB) food industry reactor (ANDRADE et al., 2020; ATASOY; EYICE; CETECIOGLU, 2020; GLANPRACHA et al., 2018; NOVAK et al., 2013). Total Solids (TS) and Volatile Total Solids (VTS) were 69.57 ± 1.45 mg·mL^{-1} and 47.52 ± 0.60 mg·mL^{-1}, respectively. The inoculum was placed in an incubator at 37 ± 2 °C to remove background organic matter for five days (ERYILDIZ; LUKITAWESA; TAHERZADEH, 2020; YANG et al., 2023).

3.2.3 Experimental set up and operation condition

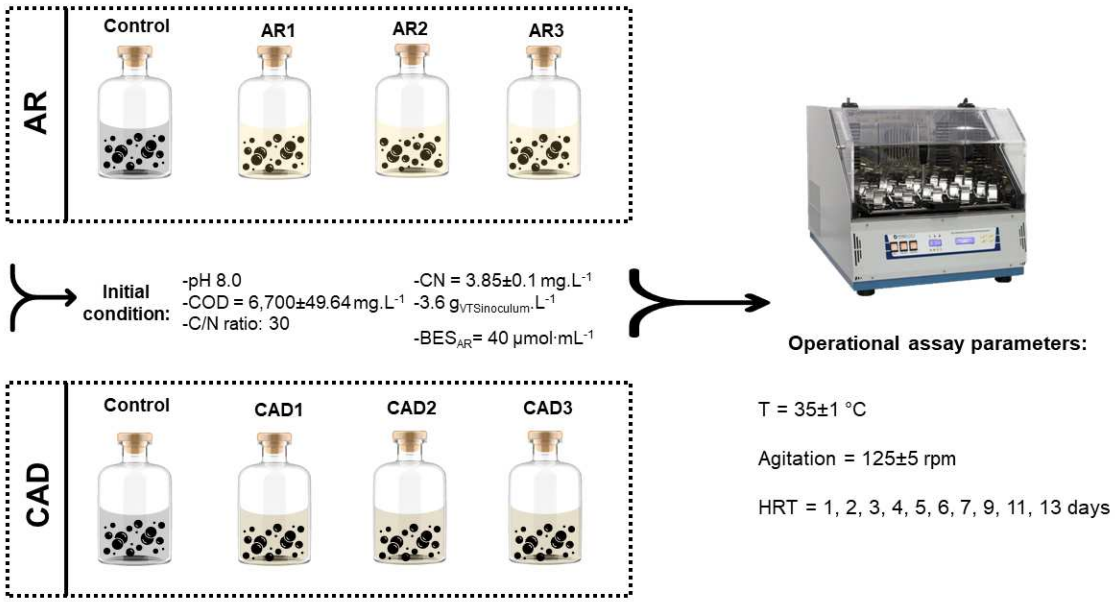
In the experimental assay 100 mL serum bottle were used, with a *headspace* of 50 mL. The serum bottle were sealed and purged with N_2 for 5 min (DAHIYA et al., 2015; LUO et al.,

2010). The AD process was performed in a benchtop shaker incubator (Tecnal®, model TE-420) for 13 days, following the literature recommendations (CREMONEZ et al., 2021; DA ROS et al., 2020; SEKOAI et al., 2021). Excessive agitation can induce stress and destruction in microorganisms and medium oxidation (MARTINEZ-BURGOS et al., 2021). Therefore, agitation was set at 125 ± 5 rpm (CHATURVEDI et al., 2019; KHATAMI et al., 2021). Temperature set at 35 ± 1 °C (HUANG et al., 2021).

Methanogenic *archaea* inhibition was performed with BES at a concentration of 40 μmol·mL^{-1} (KOSSE; LÜBKEN; WICHERN, 2016). The initial concentration of COD (6,700 ± 49.6 mg·L^{-1}) and CN (3.85 ± 0.1 mg·L^{-1}) in the reactors was adjusted to prevent the microorganisms inhibition. The used inoculum concentration was 3.6 g·L$^{-1}_{VST}$, corresponding to a 1.44 ratio (5.20 g·L$^{-1}_{VSTsubstrate}$ / 3.6 g·L$^{-1}_{VSTinoculum}$) (WANG et al., 2015a). Because AD is linked to a C/N ratio in the range of 20 to 35, and the CWW nitrogen concentration is low, yeast extract was used to achieve the C/N:30 (PANICHNUMSIN et al., 2010, 2012). Since pH influences the HCN volatilization, the pH was set to 8.0, minimizing the HCN release into the gaseous phase (DAS; SANTRA, 2011; MARTÍNKOVÁ et al., 2023). The pH adjustment was performed with sodium bicarbonate ($NaHCO_3$), in order to also act as a buffer and electron acceptor for hydrogenotrophic methanogens and hydrogenotrophic homoacetogens (GUPTA; AHAMMAD; SREEKRISHNAN, 2016). Each reactor (AR and CAD) was performed in triplicate, using inoculum and saline water as a control. Control reactors were used as a blank, i.e., the results of the biogas production, VFA, sCOD, nitrate and ammoniacal nitrogen were subtracted from the assay. The experiment was carried out in sacrificial flasks, i.e., every day 3 flasks of each condition, were taken from the incubator for analysis and disposed. The assays operational conditions are presented in **Figure 05**. Briefly, CN removal was performed in acidogenic (AR), and complete anaerobic digestion (CAD) batch reactors as described below:

- AR were carried out inhibiting the methanogens activity by the addition of 40 μmol·mL^{-1} BES; and
- CAD reactors were carried out with complete anaerobic digestion activity (without BES).

Figure 05 - Conditions operations of assay.



Footnote: AR - reactor added BES; AR1, AR2 and AR3: replicates; CAD – reactor without BES; CAD1, CAD2 and CAD3, replicates; COD - chemical oxygen demand; CN – cyanide; BES - 2-bromoethanesulfonate acid sodium salt. Source: From the author.

The parameters measured in the assays were: CN concentration, COD, ammoniacal nitrogen (NH_4^+), Nitrate (NO_3^-); pH variation; and biogas and VFA production. VFA, CN, COD, pH, (NH_4^+), and (NO_3^-) during 13 days. Cumulative biogas production was analyzed every 12 hours. Biogas production was expressed in volume at Normal Temperature and Pressure (NTP), calculated according to Equation 1 described below:

$$V_N = \frac{V_b \times (P + P_w) \times T_0}{P_0 \times T} \qquad \text{Equation 1}$$

Where V_N is the volume of the gas in the normal state (mL). V_b is the gas volume as read off (mL). P is the gas phase pressure at the reading time (mbar). P_W is the water vapor pressure as function of the ambient temperature (mbar). T_0 is the normal temperature (273 K). T is the gas temperature in K. P_0 is the normal pressure (1013 mbar).

3.2.4 Analytical methods

The CN determination was performed by colorimetric methodology (DROCHIOIU, 2002b; SURLEVA; DROCHIOIU, 2013). Briefly, the samples were centrifuged in 5,000 x g (4 °C) for 15 min, the supernatant was filtered through a membrane (0.22 µm, PES), then this

sample (1 mL) was treated with ninhydrin solution (28 mM) (0.5 mL) at room temperature. It was read at 494 nm using a 1 cm quartz cuvette (Agilent, 6610001100), in a spectrophotometer (Hach, model DR 5000). The standard curve was performed using KCN in the range of 20-120 ng·mL^{-1} (**Figure S11**).

The total sugars were determined according to a colorimetric method described by DuBois et al., 1956. COD was determined in two techniques, in its soluble (sCOD) and total (tCOD) forms, (APHA, 2005). To determine the sCOD, the samples were centrifuged at 5,000 x g (4 °C) for 10 min and taken its supernatant. Meanwhile, to determine tCOD the samples analyze without centrifugation. NH_4^+ determination was performed by the Nessler method (APHA, 2005; JEONG; PARK; KIM, 2013). NO_3^-, COD, Total Solids (TS), Total Fixed Solids (TFS), Total Volatile Solids (TVS), Dissolved Total Solids (DTS), Dissolved Fixed Solids (DFS), Dissolved Volatile Solids (DVS), Total Suspended Solids (TSS), Fixed Suspended Solids (FSS), and Volatile Suspended Solids (VSS) were determined according to APHA, (2005). For DTS, DFS, DVS, TSS, FSS and VSS were centrifuged at 5,000 x g (4 °C) for 10 min.

The TOC was performed in the TOC-VCPH analyzer (Shimadzu). The liquids samples were centrifuged at 5,000 x g (4 °C) for 10 min and filtered at 0.45 μm cellulose acetate (CA) membrane filter. Before the measurement, the samples were neutralized with an HNO_3 solution (0.01 M). The analysis is based on the combustion catalytic oxidation method at 680 °C, coupled with a non-dispersive infrared detector. Total Nitrogen Kjeldahl (TNK) was carried out according to Amin et al. (2004). Total acidity analyzed (ABNT, 2015). Electric conductivity and pH were determined in a benchtop pHmeter (Quimis®, model Q402M). Starch concentrations were analyzed by Total Starch Kit (K-RSTAR, Megazyme Co.) following recommendations (KOAKUZU et al., 2015).

The VFA concentration was determined by Ultra-Fast Liquid Chromatography (UFLC) using the Shimadzu Prominence LC-20A (Shimadzu, Tokyo, Japan) chromatograph with a Bio-rad® Aminex HPX-87H (300 x 7.8 mm) column, and refractive index detector (RID-20A) at 40 °C. The samples were previously centrifuged at 5,000 x g (4 °C) for 15 min, filtered (0.22 μm, PES), and transferred to the vials. 10 μL injection of the sample was eluted with 5 mmol·L^{-1} of H_2SO_4 in a flow rate of 0.5 mL·min^{-1}. Oven temperature was maintained at 35 °C and run time was 42 min. Concentrations were calculated from the standard curve of acetic, butyric, formic, propionic, and valeric acid. Biogas accumulation was measured using a ground glass syringe (XIE et al., 2023). The biogas composition (CH_4, CO_2, H_2) was determined using

gas chromatograph (Shimadzu, model GC-2014ATFSPL) equipped with detector FID and TCD (current 45 mA), and column 60/80 Carboxen-1000 (Supelco, serial number 12390-U). 300 µL of the sample was inserted into the injector at a temperature of 210 °C using 30 mL·min^{-1} of argon (99.999 %) as carrier gas, in the run time of 16.5 min. Initial oven temperature at 180 °C, increasing 1 °C·min^{-1} to 210 °C maintained by 2.5 min. For more details on the procedure, see da Silva et al. (2022). All analyzes were performed in at least three replicates. Figures were obtained with Origin 9.9.5 (OriginLab Corp., USA).

3.2.5 Microbial community analysis

The microbiology community was realized by 16S rRNA gene amplification and sequencing. At the beginning and end of the experiments (t_0 and t_f, respectively), the biomass was centrifuged at 5,000g at 4°C for 10 min. Then, the samples were frozen at −20 °C and sent to the Neoprospecta Laboratory (Florianópolis city, Brazil) for analysis according to the methodology (CHRISTOFF et al., 2017). Briefly, the DNA extraction was carried out following a protocol (Neoprospecta *Microbiome* Technologies, Brazil), details of which are subject to intellectual property rights. The DNA was quantified on a Qubit fluorimeter with the dsDNA BR assay kit (Invitrogen, Waltham, MA, USA). After quantification, the DNA was diluted to 0.5 ng µL^{-1} and stored at −20 °C for molecular analyses. Amplification was performed with the specific primers for the V3–V4 region of 16S rRNA, 341F with the sequence (5′-CCTACGGGRSGCAGCAG-3′), and 806R with the sequence (5′-GGACTACHVGGGTWTCTAAT-3′) (CAPORASO et al., 2012; WANG; QIAN, 2009). The libraries were sequenced through the MiSeq Sequencing System (Illumina Inc., San Diego, CA, USA) using standard primers supplied by the manufacturer with 500 cycles and *paired-end* sequencing. The sequences were analyzed using the Sentinel *pipeline*. The bioinformatics pipeline performs the analyzes on the proprietary database (Neoprospecta *Microbiome* Technologies, Brazil). The sequence database for the 16S rRNA and ITS genes contains complete gene sequences retrieved from unambiguous genomes and filtered for chimera sequences.

3.3RESULTS AND DISCUSSION

3.3.1 CWW characterization

The cassava wastewater (CWW) physical-chemical characterization (see **Table 05**) showed that it has a high organic concentration (81,730 ± 534 mg $O_2 \cdot L^{-1}$), corroborating with the literature (ANDRADE et al., 2020; COUTINHO RODRIGUES et al., 2021; VIANA; DÜSMAN; VICENTINI, 2014). The amount of starch (0.46 ± 0.01 $g \cdot 100g^{-1}_{dry\ matter}$), one of the major contributors to the high concentration of organic matter, is available for easy hydrolysis due to being in a soluble form (see **Table 05**), thus enabling the AD process, corroborating other studies (ANUPONG et al., 2022; THANGAVELU et al., 2020).

Table 05 - Substrate characterization

Parameter	CWW
pH	4.75 ± 0.13
EC ($\mu S \cdot cm^{-1}$)	6,389.5 ± 33.62
Total Acidity ($mg \cdot KOH \cdot g^{-1}$)	3.31 ± 0.02
Free Total Cyanide ($mg \cdot L^{-1}$)	139.75 ± 5.40
Total Starch ($g \cdot 100g^{-1}_{DM}$)	0.46 ± 0.01
Soluble Starch ($g \cdot 100g^{-1}_{DM}$)	0.42 ± 0.01
tCOD (mg $O_2 \cdot L^{-1}$)	81,730 ± 534
sCOD (mg $O_2 \cdot L^{-1}$)	73,411 ± 816
Total Solids	59.08 ± 2.24 $mg \cdot g^{-1}$
	59.14 ± 1.95 $g \cdot L^{-1}$
Total Fixed Solids	7.06 ± 0.16 $mg \cdot g^{-1}$
	7.08 ± 0.14 $g \cdot L^{-1}$
Total Volatile Solids	52.02 ± 2.15 $mg \cdot g^{-1}$
	52.08 ± 1.90 $g \cdot L^{-1}$
Dissolved Total Solids	52.41 ± 2.0 $mg \cdot g^{-1}$
	52.67 ± 1.67 $g \cdot L^{-1}$
Dissolved Fixed Solids	6.77 ± 0.2 $mg \cdot g^{-1}$
	6.77 ± 0.15 $g \cdot L^{-1}$
Dissolved Volatile Solids	44.43 ± 4.32 $mg \cdot g^{-1}$
	44.64 ± 4.10 $g \cdot L^{-1}$
Total Suspended Solids	7.22 ± 0.33 $mg \cdot g^{-1}$
	7.30 ± 0.30 $g \cdot L^{-1}$
Fixed Suspended Solids	0.26 ± 0.02 $mg \cdot g^{-1}$
	0.26 ± 0.025 $g \cdot L^{-1}$
Volatile Suspended Solids	6.66 ± 0.04 $mg \cdot g^{-1}$
	6.77 ± 0.07 $g \cdot L^{-1}$
TOC ($mg \cdot L^{-1}$)	31,033 ± 655.77
TKN ($mg \cdot L^{-1}$)	567.8 ± 99,21
C/N ratio	54
Total sugar ($mg \cdot L^{-1}$)	714.2 ± 97

Footnote – CWW = cassava wastewater; EC = electric conductivity; DM – dry matter; tCOD = total chemical oxygen demand; sCOD = soluble chemical oxygen demand; TOC = total organic carbon; TKN = Total Kjeldahl Nitrogen; DM = dry matter; WM = wet matter; TSS = total suspended solids. Source: From the author**.**

The sCOD (73,411 ± 816 mg $O_2 \cdot L^{-1}$) covers a large part of the organic matter, suggesting that the effluent is easily biodegradable. Comparing with other studies (1,730 – 38,210 mg $O_2 \cdot L^{-1}$), the sCOD value was significantly higher (COLIN et al., 2007; LU et al., 2019; ZHANG et al., 2011). The tCOD value in CWW in the present study is high (81,730 ± 534 mg $O_2 \cdot L^{-1}$), with >90% (sCOD) already available for easy biodegradation. In other studies, COD is very variable (6,014 – 24,000 mg $O_2 \cdot L^{-1}$) (FLECK et al., 2019; FRANCISCO et al., 2015; JIRAPRASERTWONG; MAITRIWONG; CHAVADEJ, 2019).

It is found in the literature that TVS from CWW comprise a large portion of the TS composition, as presented in the **Table 05**. Volatile solids contribute to increase the organic concentration of the effluent (FERRAZ; BRUNI; DEL BIANCHI, 2009). In the present study, the CWW presented TS and TVS of 59.14 and 52.08 $g \cdot L^{-1}$, respectively, therefore TVS represents 88.06 % of the TS. Similar values were observed in the study of Carraro et al. (2021). Intrinsic characteristics of CWW to high acidity and low pH (CREMONEZ et al., 2021). In the present study, low pH (4.75 ± 0.13) was observed, corroborating with the literature (ANDRADE et al., 2020; ANNACHHATRE; AMORNKAEW, 2001; ANUPONG et al., 2022; FLECK et al., 2019; SUN et al., 2012; THANGAVELU et al., 2021). The observed electrical conductivity value (6,389.5 ± 33.62 $\mu S \cdot cm^{-1}$) was relatively high, but similar with other studies (NEVES et al., 2017; PRASERTSUNG; REUNGSANG; RATANATAMSKUL, 2012; THANGAVELU et al., 2020), probably due to the total dissolved ions in the composition of CWW (MCCLESKEY et al., 2012; MCNEIL; COX, 2000; OYEWUSI; OSUNBITAN; TAIWO, 2021).

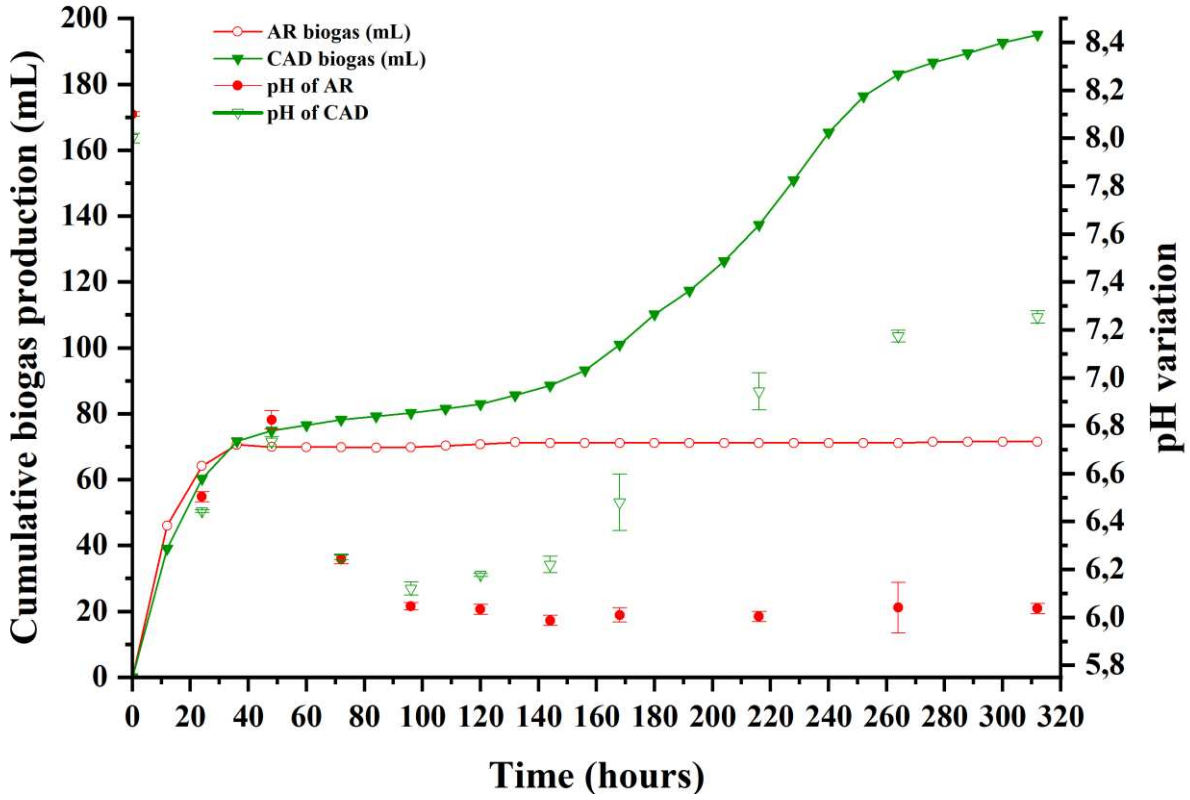
The TOC value found in this study (see **Table 05**) was similar to the result observed (27,675 ± 11,719.1 $mg \cdot L^{-1}$) by Costa et al. (2021). Furthermore, close values were obtained by Prasertsung et al. (2012). However, Carraro et al. (2021) found a considerably lower value (i.e., 1 $mg \cdot L^{-1}$) than the present study, as CWW characterization may differ due to the analysis technique, industry collected or cassava species. The nitrogen content (TKN) was low compared to the high carbon concentration, being a factor correlated to the CWW, influencing the C/N ratio, corroborated by the literature (KAEWKANNETRA et al., 2009; PRASERTSUNG; REUNGSANG; RATANATAMSKUL, 2012; QUAN et al., 2014). Furthermore, a high C/N ratio (i.e., 54.65), also reported by Francisco et al. (2015), was observed in the present study, suggesting nitrogen supplementation to achieve the appropriate C/N ratio (20-35) for AD (PANICHNUMSIN et al., 2010, 2012).

The CN concentration present in the CWW of this study was high (139.75 ± 5.40 $mg \cdot L^{-1}$) demonstrating the high toxicity of the effluent in the environment. However, this value (102 – 252.66 $mg \cdot L^{-1}$) is consistent with the literature (ADEMAKINWA; AGUNBIADE; FAGBOHUN, 2021; GUSMÃO et al., 2018; POTIVICHAYANON et al., 2020). Although, some studies present lower values (2.30 – 89.89 $mg \cdot L^{-1}$) (ANDRADE et al., 2020; COUTINHO RODRIGUES et al., 2021; OYEWUSI; OSUNBITAN; TAIWO, 2021). The CN concentration variation in CWW is evident. Moreover, the values presented in the literature for some parameters are different from those of the present study, but the values found are due to many possibilities, such as industry technology, country, age of the cassava plant, cassava variety, and precipitation (ADEMAKINWA; AGUNBIADE; FAGBOHUN, 2021; CARDOSO et al., 2005; HASAN et al., 2015; RAY et al., 2015; SÁNCHEZ et al., 2014; SANTISOPASRI et al., 2001).

3.3.2 Effect of CWW on biogas production and accumulation of VFA

The cumulative biogas production was similar to AR and CAD in the first three days (see **Figure 06**). From the 2nd day, AR did not produce biogas, probably due to the pH value and the inhibitory effect on methanogens. In addition, on the 1st day, the pH in the AR was drastically reduced (**Figure 06**), due to the organic matter conversion into acids. The cumulative biogas production was constant in AR from the 2nd day onwards, due a large part of the complex organic matter was converted into VFA and H_2 (**Figure 06**). Between the 4th and 6th day, there was a small increase in production (1.7 mL), probably due to the pH being in a suitable condition for H_2-producing microorganisms, such as *Clostridium butyricum* (BECKERS et al., 2010; MASSET et al., 2010).

Figure 06 - Cumulative biogas production (mL) and pH variation



Source: From the author.

Apparently, the inhibition action of CN to methanogens (CAD) occurred until the 5th day (120 hours) (**Figure 06**), unlike observed by Glanpracha et al. (2018) which required only 3 days. In addition, on the 5th day the CN was almost completely degraded, and pH and biogas production started to increase. Evaluating the between 72 hours (3 days) and 144 hours (6 days) the biogas production was relatively constant, and the pH varied among 6.12-6.25 (**Figure 06**), indicating that there was inhibition in the biogas production due to the excess of acids because CN may have inhibited the methanogens activity. In another study, using influent with a 5 $mgCN{\cdot}L^{-1}$ concentration, there was no influence on the cumulative biogas production, diverging in the present study (NOVAK et al., 2013). Probably due to the mixed culture of microorganism from brewery wastewater, used as inoculum, having the ability to tolerate and degrade CN.

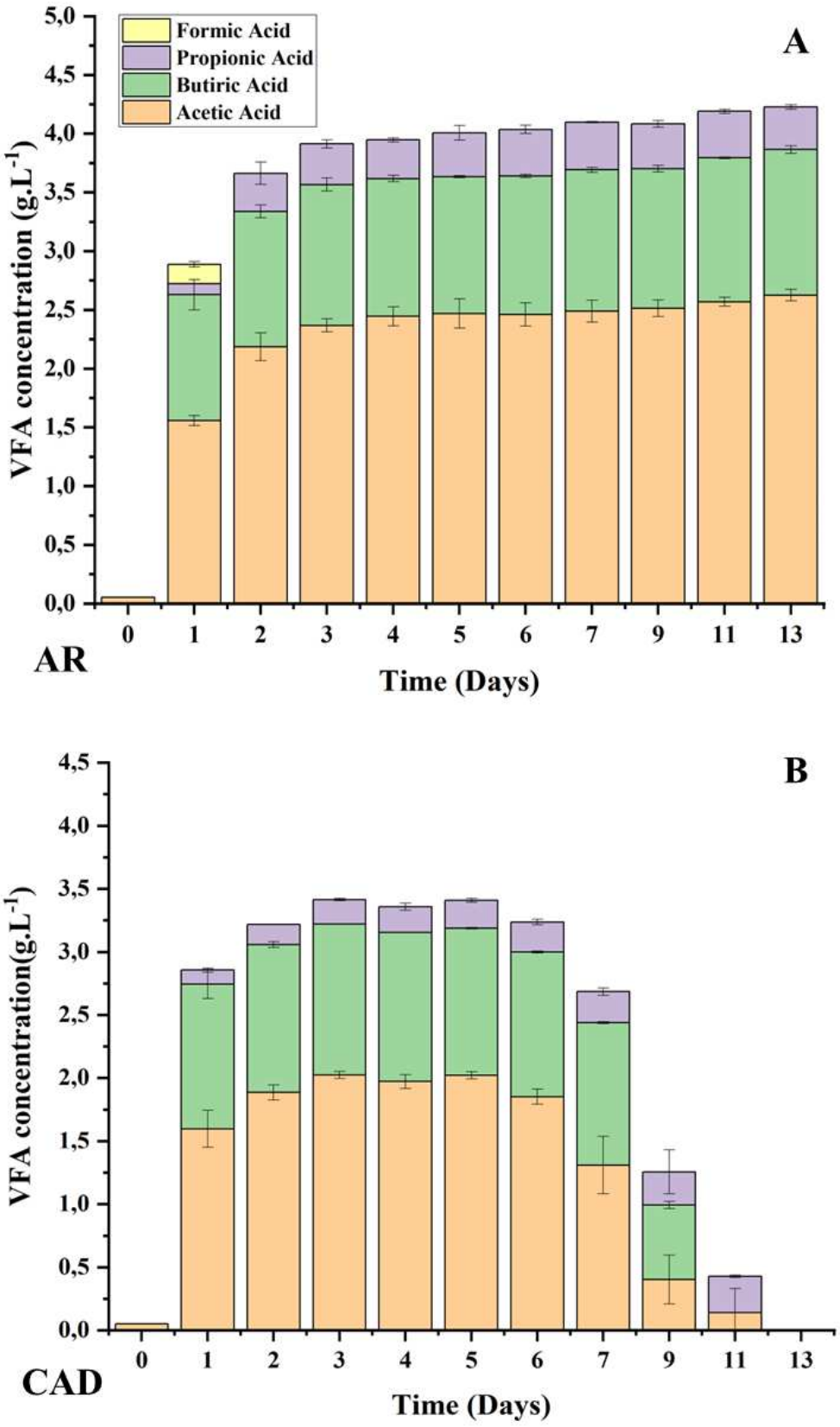
The biogas composition in the final assay (**Figure S12**) was performed to verify the by-product concentrations produced in the reactors. When analyzing the AR, the CH_4 concentration was <1 %, and H_2 >44 %, confirming the inhibitory effect on methanogens. A similar result was reported by Kosse et al. (2016), who performed biochemical methane potential tests (BMP) for 15 days obtaining a CH_4 concentration in the range of 0.99 to 1.84 %.

In another study at 12-hour HRT a H_2 concentration of 29.51 ± 1.12 % was reported (THANWISED; WIROJANAGUD; REUNGSANG, 2012). In addition to the methanogens inhibition in AR, hydrogen production seems to be related to the rapid acids production, corroborating with the literature (KHANAL, 2003; RAMKUMAR et al., 2021). The CH_4 concentration (i.e., 74 %) found in CAD was similar to the literature (GLANPRACHA et al., 2018; LI et al., 2019; NOVAK et al., 2013). However, in CAD the H_2 concentration was low (i.e., 0.54 %) (see **Figure S12**), probably due to the ammonia concentration in the medium (see **Figure 08**), as acetoclastic methanogens are sensitive to increased ammonia contractions (YENIGÜN; DEMIREL, 2013; YI et al., 2023). Another factor for the low H_2 production is the non-inhibition of BES in the activity of hydrogenotrophic methanogens.

The analysis of VFA production showed that acetic acid was the major acid, followed by butyric and propionic acid, in both AR and CAD (**Figure 07**). Furthermore, the behavior of acid production helped to understand the inhibition of AD by the CN in the first 5 days. According to **Figure 07-A**, (AR) in the day one, there was an intense production of acetic acid. During the entire time of the AR experiment, acetic acid had the highest yield, followed by butyric acid. Likewise, a high yield of acetic acids in pH range of 5 to 9 has been reported (CUBERO-CARDOSO et al., 2022). The maximum concentration achieved for acetic acid was 2.62 $g{\cdot}L^{-1}$ on the 13th day, while for butyric acid it was 1.23 $g{\cdot}L^{-1}$ also on the 13th day. Butyric acid production (**Figure 07-A**) was the same as reported in the literature (KOSSE; LÜBKEN; WICHERN, 2016).

The decrease in pH (**Figure 06**) was due to the increase in VFA concentration in the reactors. Other studies reported a decrease in pH with increasing VFA concentration (KOSSE; LÜBKEN; WICHERN, 2016; VALENTINO et al., 2021). **Figure 07-A** shows that in 1-day, the formic acid concentration was 0.16 ± 0.02 $g{\cdot}L^{-1}$, but in **Figure 07-B** it was not detected. CN hydrolysis can generate HCOOH. According to Zaher et al. (2006), HCOOH can be used to carry out the methanogenic pathway by microorganisms, such as the genus *Methanosarcina*. In addition, cytochrome-free methanogens generate CH_4 by reduction of CO_2 with electrons derived from H_2, HCOOH, or secondary alcohols (MAND; METCALF, 2019). This fact is corroborated when observing **Figure 07-B**, which formic acid was not identified during fermentation. It can be seen that in the 1st day (**Figure 07-A)**, formic acid was no longer detected, probably the BES concentration was not enough to inhibit all methanogens. In addition, the high VFA production is attributed to the soluble substance generation and the methanogens inhibition (LIU et al., 2014).

Figure 07 - VFA production and consumption kinetics during the assays



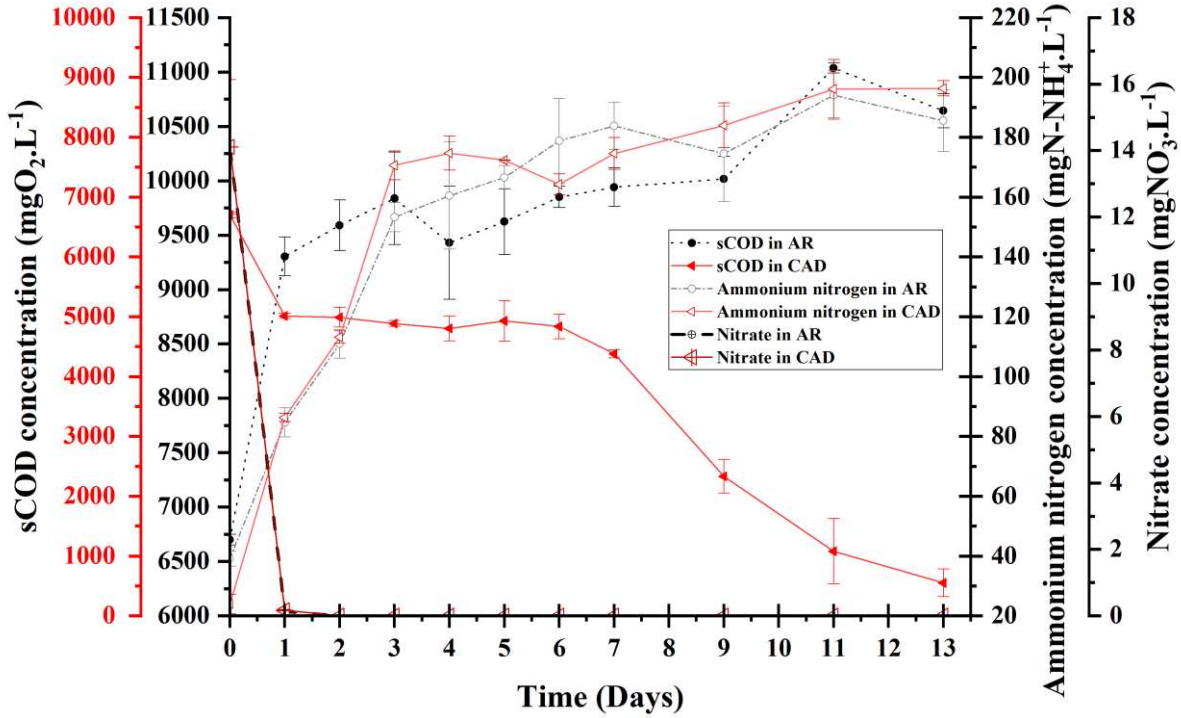
Source: From the author.

3.3.3 Organic matter and nitrogenous compounds

In AR, sCOD had a considerable increase on the first day (**Figure 08**). Continuing with an increase until the third day and a small decrease on the fourth but considering the standard deviation it is within the tolerable range. The increase in sCOD was similar to that observed by Ma et al. (2016) in R3 (untreated sludge, pH = 10.0). In AD, sCOD plays an important role linking hydrolysis and acidogenesis, influencing the VFAs yield (ZHOU et al., 2018). Organic acids can accumulate in the medium due to disturbances (e.g., high organic load) to methanogenic microorganisms, preventing the conversion of acids to biogas (SUN et al., 2020). In CAD, sCOD had a slight decrease of 25.19 % on the 1st day of fermentation (**Figure 08**). Until the 6th day, efficiency remained constant, probably due to CN inhibition and microorganisms adaptation. The sCOD in the initial time in both reactors (AR and CAD) was 6,700 ± 49.6 mg·L^{-1}. Nonetheless, the efficiency removal of sCOD in CAD was 91.78 %.

The increase in NH_4^+ concentration in AR and CAD (166 and 170 mg·L^{-1}, respectively) was noticeable from the 3rd day (**Figure 08**). This phenomenon is due to the proteins breakdown into amino acids during AD and the low pH (see **Figure 08**), which can modify the ammonium phase balance, consequently increasing ammonium (MLINAR; WEIG; FREITAG, 2022; SHENG et al., 2013; XIAO et al., 2022; YELLEZUOME et al., 2022; YENIGÜN; DEMIREL, 2013). In addition, during the CN hydrolysis by-products, such as HCOOH and NH_4^+, are obtained (GLANPRACHA et al., 2018). Apparently, from the 6th day on, the ammonium ion concentration did not affect the biogas producing microorganisms. Although bacteria are affected by NH_4^+, *archaea* are not affected, because they have a more robust membrane (JAIN, 2014; MLINAR; WEIG; FREITAG, 2022). Moreover, NH_4^+ can be used as a source of nitrogen for bacterial growth (GLANPRACHA et al., 2018; ZAHER et al., 2006). Inferring the explanation consumption ammonium ion in AR and CAD, on the 9th and 6th day, respectively (**Figure 08**).

Figure 08 - Concentration of organic matter (as COD) and nitrogenous compounds (as nitrate and ammonium nitrogen) concentrations in the reactors as a function of time (days).



Footnote: sCOD – soluble chemical oxygen demand. Source: From the author.

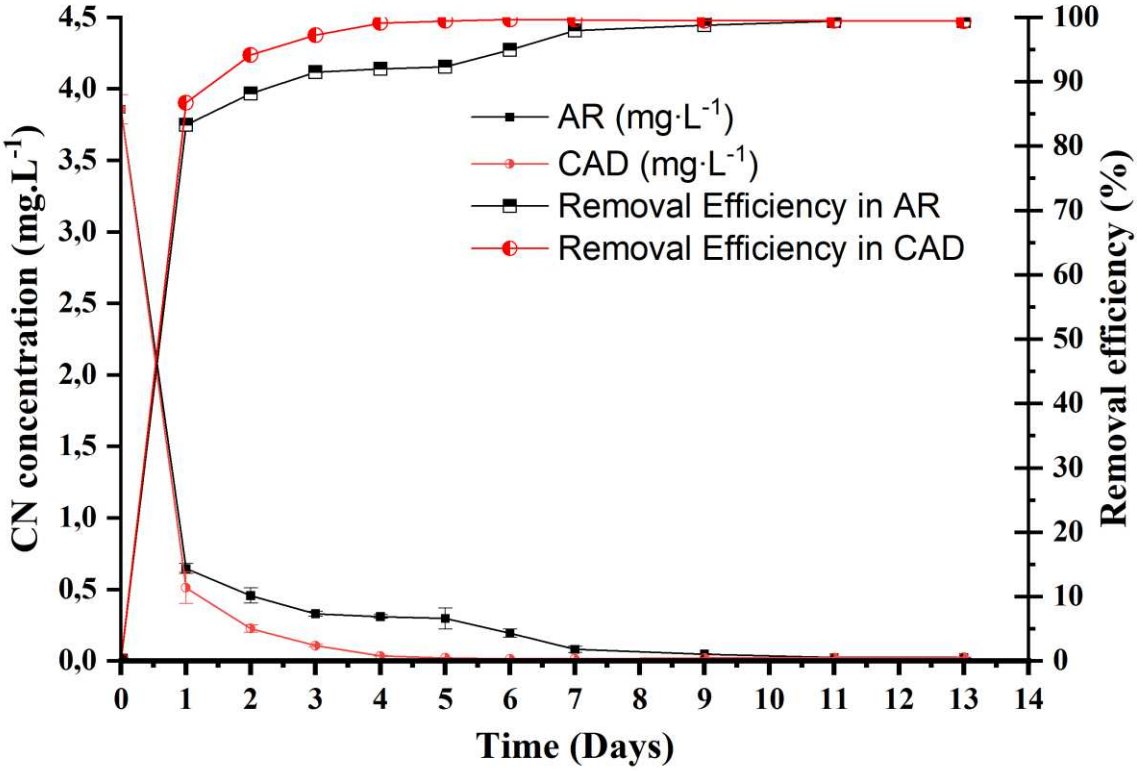
3.3.4 Comparison of CN removal on AR and CAD batch reactors

The CN removal in both reactors, AR and CAD, on the first day was 83.26 and 86.73 %, respectively (**Figure 09**). From the 1st to the 6th day, the removal efficiency was slightly different between the reactors. In this period, the CN concentration in the 1st day was 0.64 mg·L^{-1}, achieving removal of 69.88 % until the 6th day in AR. Nevertheless, for CAD in the same period the CN removal reached 97.27 %. Suggesting that the methanogenic microorganisms on AD might quickly degrade the CN. Contrasting with the study by Kaewkannetra et al. (2011) that used microbial fuel cells (MFC), a CN removal efficiency of 70 % was reported. Similarly, in another study using *Azotobacter vinelandii* reached 69.7 % CN removal in fermentation 66 hours (KAEWKANNETRA et al., 2009). Notwithstanding, the presence of microorganisms capable of tolerating and CN degrading in the present study is corroborated by the studies previously described. Another interesting aspect from the 5th to the 7th day AR reduced CN by 73.05 %, achieving similar the CAD removal efficiency on the 7th day. From day 8 onwards, CN removal was >98 % for both reactors (**Figure 09**).

The maximum CN removal efficiency of the process was 99.40 % and 99.37 % for AR and CAD, respectively. However, on the 3rd day CAD had already reached 99.09% CN removal

efficiency, while AR had 91.45%. This deduces that the AD process in CAD can be stopped on the 3rd day, due to the high CN removal efficiency. Major studies using cassava waste achieved excellent results in CN removal in short period time (GLANPRACHA; ANNACHHATRE, 2016; HASAN et al., 2015; KAEWKANNETRA et al., 2009; POTIVICHAYANON et al., 2020). For example, Potivichayanon et al. (2020) used a fixed-film sequencing batch reactor (F-SBR) for removal high CN concentration using CWW. The authors reported that at 5-day HRT, an efficiency of 95.45 % in CN removal was achieved. The low HRT is an advantage, being considered in biological treatments.

Figure 09 - Daily concentration and cyanide removal efficiency in acidogenic reactor (AR) and complete anaerobic digestion (CAD) reactors.



Footnote: CN – cyanide. Source: From the author.

Some reasons for efficiencies if this process can be considered: microbiota, pH, CN concentration, temperature, ammonia, and various metal ions (ADJEI; OHTA, 2000; BAXTER; CUMMINGS, 2006; PEREIRA; ARRABAÇA; AMARAL-COLLAÇO, 1996). Temperature and pH are important parameters for CN biodegradation due to enzymes being produced in mesophilic conditions (DASH; GAUR; BALOMAJUMDER, 2009; VEDULA; DALAL; MAJUMDER, 2013). Additionally, the pH optima for bacterial growth are typically 6 to 8 and CN-degrading enzymes generally have pH optima in the range of 6 to 9. Observing

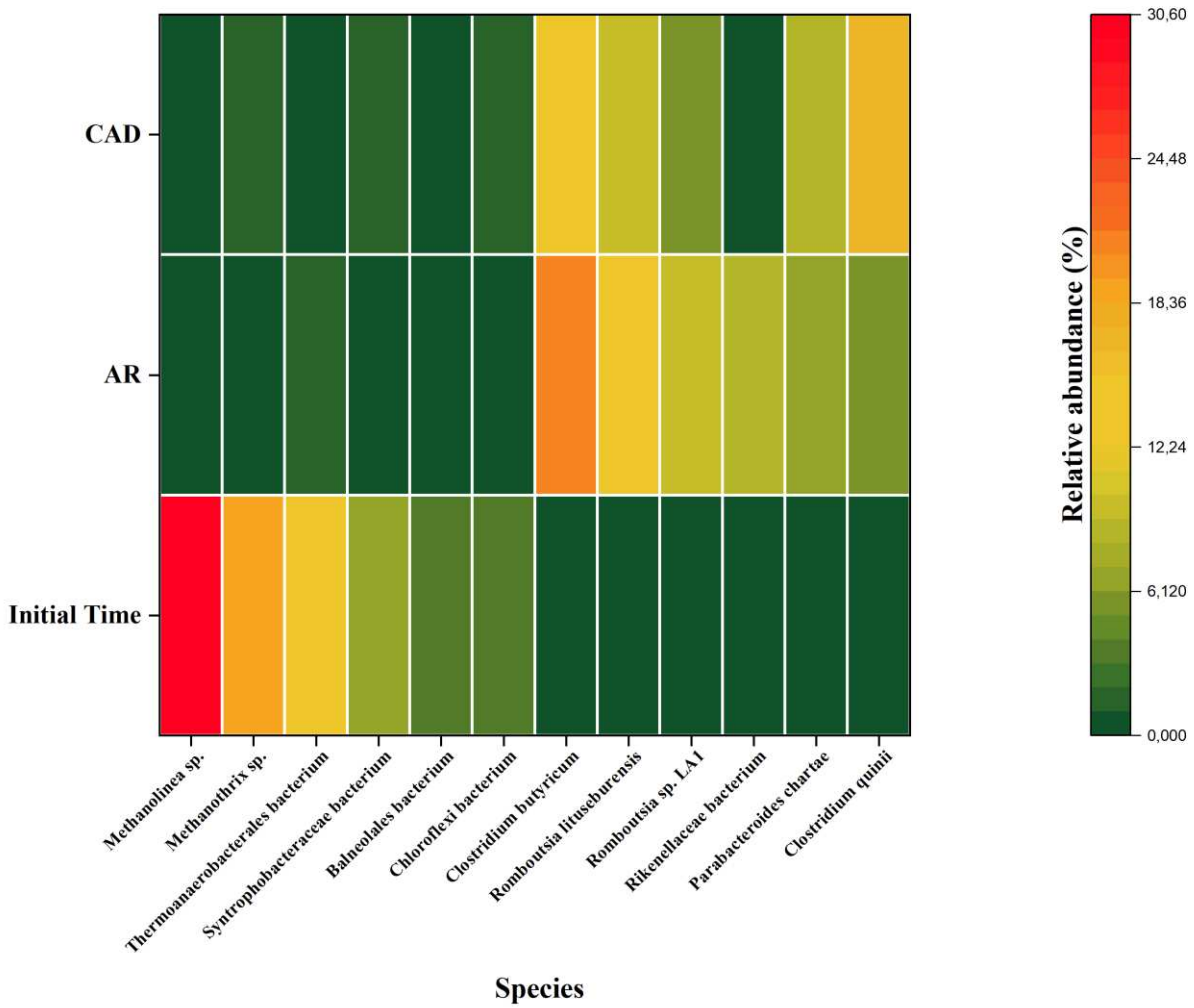
Figure 06, the pH during the experiment remained within the range (i.e., 6 to 8), suitable for the enzymes activity involved in CN degradation.

The biological process applied in the present study (AD) has some advantages over aerobic processes, such as high efficiency in CN removal, production of biogas, VFA and low energy resources. For example, Koksunan et al. (2013) obtained a high removal efficiency (75.7 %) of CN using *Azobacter vinelandii*. Furthermore, in another study using *Candida tropicalis* ASY2 achieved only 78.95 % efficiency (THANGAVELU et al., 2020). However, in another study the CN removal efficiency was even lower, only 25 % (LAWAL et al., 2019). These results corroborate that AD is more efficient for CN removal, in addition to the advantage of obtaining VAP. On the other hand, some process changes can be implemented. For future paper, the new strategies adoption may favor the process, for example: i) check the CN removal in higher concentrations and different cassava species, ii) variation in operating conditions, as temperature, pH, the concentration of organic matter, and inoculum.

3.3.5 The microbial community

The microbiota analysis allows to understand the microorganisms involved on CN degradation. In both reactors studied, microbial analysis identified two phylum in the initial ($\mathbf{t_0}$) and final ($\mathbf{t_f}$) time (Figure S13). The percentage found between *archaea* and *bacteria* was relatively different in the $\mathbf{t_0}$, that is, *archaea* accounted for 57.04 % of the sample, while *bacteria* accounted for only 42.97 %. The foremost dominating phyla in the beguining ($\mathbf{t_0}$) and in the end ($\mathbf{t_f}$) of the experiment were *Firmicutes, Euryarchaeota, Bacteroidetes,* and *Proteobacteria*. A similar result was reported by Alalawy et al. (2021) when they analyzed the microbial community of three anaerobic sludges. *Methanolinea* sp., *Methanothix* sp. and *Thermoanaerobacterales bacterium* were more abundant in $\mathbf{t_0}$ (**Figure 10**) 30.53, 19.35, and 13.20 %, respectively. In addition, *Pseudomonas stutzeri* was identified, differently from the $\mathbf{t_f}$ (**Table S6**), probably due to the antimicrobial activity of secondary metabolites excreted from other microorganisms, exercising an interference competition (JACOBSON et al., 2018; OLIVEIRA et al., 2020; ÖZÇAM et al., 2022). *P. stutzeri* is well-known as a CN-degrader suggesting that the marked removal on 1st day was due to this microorganism. (KARAVAIKO et al., 2000; NWOKORO; DIBUA, 2014; SINGH; ARORA; SACHAN, 2018).

Figure 10 - Relative abundance of microorganisms in the initial time (t_0) and in AR and CAD (t_f)



Source: From the author.

Analyzing the experiments performed with the BES addition (AR), *C. butyricum* is more abundant when compared to the initial time (**Figure 10**), probably due to the reactors pH. This is also corroborated by the production of butyric acid (see **Figure 07**) and hydrogen since the species can produce (MASSET et al., 2010; SZYMANOWSKA-POWAŁOWSKA; ORCZYK; LEJA, 2014). *Clostridium* and *Pseudomonas,* genus were identified at the final and initial time, described as CN-tolerant groups (GUPTA; SREEKRISHNAN; SHAIKH, 2018).

The CAD experiments had greater microbiological diversity (**Table S6**). Furthermore, according to the **Figure 10**, *Clostridium quinni* was more abundant under these conditions. *C. quinii* utilize glucose and other sugars to produce H_2, CO_2, acetate, ethanol, and butyrate at pH around 7.4 and mesophilic conditions (SVENSSON et al., l1992). The genus *Citrobactler* in a small proportion was observed in the final experiments (**Figure 10**). Patil and Paknikar, (2000)

used *Citrobacter* spp. to degrade CN and found the CN-complexes and metal-CN removal being the first study to report this observation. Therefore, the reduction methanogens in CAD was probably due to the initial CN concentration and pH reduction (CHEN; DU; XIE, 2021). In addition, results showed that the methanogens are sensitive to CN-inhibition (FEDORAK; ROBERTS; HRUDEY, 1986; GUPTA; SREEKRISHNAN; SHAIKH, 2018).

The microbiological analysis provided a better understanding of the biological process. In addition, it allows the observation of changes in the microbiota, being able to infer the final products (CH_4, H_2 and VFA) obtained from the reported microorganisms. The change in the microbiota during AD is reported in other studies (CHE et al., 2021; JUNG et al., 2013).

3.4 CONCLUSIONS

It is evident that cassava wastewater is rich in organic matter and toxic compounds. Intrinsic characteristics (pH, COD, BOD, CN, solids, C/N ratio) will vary by cassava species, industry, region, and climatic conditions. The CN concentration is extremely variable and a parameter that deserves attention due to the damage caused to the environment. In the present study, two-stage AD reactors (AR and CAD) were used to remove CN and obtain VAP. Many studies demonstrate good CN removal efficiency results in a few days of treatment, but in the present study, CN removal was accentuated as early as the 1st day, reaching >83%, both in the acidogenic process (AR) and in complete digestion (CAD). The results demonstrate that CN removal from CWW can be feasible both in acidogenic and complete anaerobic digestion processes. The process choice will depend on the product of interest to be generated, and the technology available for recovering these final products. The VFA concentration was very pronounced and constant in AR due to the methanogens inhibition. It was found a higher yield for acetic acid followed by butyric acid. The cumulative biogas production in CAD was low for 5 days, reaching an inflection point on the 11th day. *Clostridium butyricum* and *C. quinni* were more abundant in AR and CAD, respectively. For future paper, the new strategies adoption may favor the process, for example: check the CN removal in higher concentrations and different cassava species, variation in operating conditions, such as temperature, pH, concentration of organic load and inoculum.

Chapter

4

Conclusion and suggestions for future studies

In this chapter, there are presented the conclusions and the suggestion to suggestions for future studies.

4 CONCLUSIONS AND SUGGESTIONS FOR FUTURE STUDIES

4.1CONCLUSIONS

In chapter 2, the literature review showed that the waste production in the cassava industry is mostly divided into solids and liquids. These waste contain a large amount of organic matter with the potential to obtain products of interest. In addition, the high cyanide concentration in these residues, resulting from the enzymatic hydrolysis of linamarin and lotaustralin, is evident. Some cyanide determination methods are more advantageous, such as direct colorimetric methods. The generation of cassava-related waste starts at the plantation, covering each stage of the production process. This several amount of waste containing these compounds must be treated in order not to cause a negative environmental impact. Among the treatment processes, biological ones stand out due to the use of cyanide by microorganisms as a source of carbon and nitrogen, in addition to producing biogas, VFA and other products of interest. In biological processes, five cyanide degradation and/or conversion pathways are described. The adverse effects of cyanide on methanogens are evident, however with adaptation of the mixed culture, high concentrations of cyanide can be biodegraded using traditional biological processes. Other biological processes must be applied for the degradation of toxic compounds, such as cyanide, present in cassava wastewater.

In chapter 3, exposure of anaerobic mixed culture to cyanide present in the cassava effluent proved to be effective in removing >83 % on the first day. The acidogenic reactors (AR) achieved similar removal efficiency with the complete digestion reactors (CAD). Despite the inhibition of biogas production in the initial days, good yields in VFA and H_2 production were observed. In addition, microorganisms able to tolerate and degrade cyanide were identified at the beginning and end of the assay. Methanogens Inhibition was responsible for the increase in sCOD in RA. On the other hand, CAD obtained removal above 90% of sCOD.

4.2 SUGGESTIONS FOR FUTURE STUDIES

Some strategic measures can be adopted to improve knowledge, optimize processes and test other conditions to confirm cyanide biodegradation, as described below:

- Evaluate the CN removal from CWW in higher organic load and consequently higher CN concentration.

- Evaluate the influence of other operational conditions, such as temperature and pH.
- Perform the CN removal from CWW in a continuous reactor.
- Cassava effluent treatment with high cyanide concentration using microbial fuel cell (MFC).

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SUPPLEMENTARY MATERIAL

CHAPTER 3 - SUPPLEMENTARY MATERIAL

Figure S11 - Calibration curve for cyanide determination.

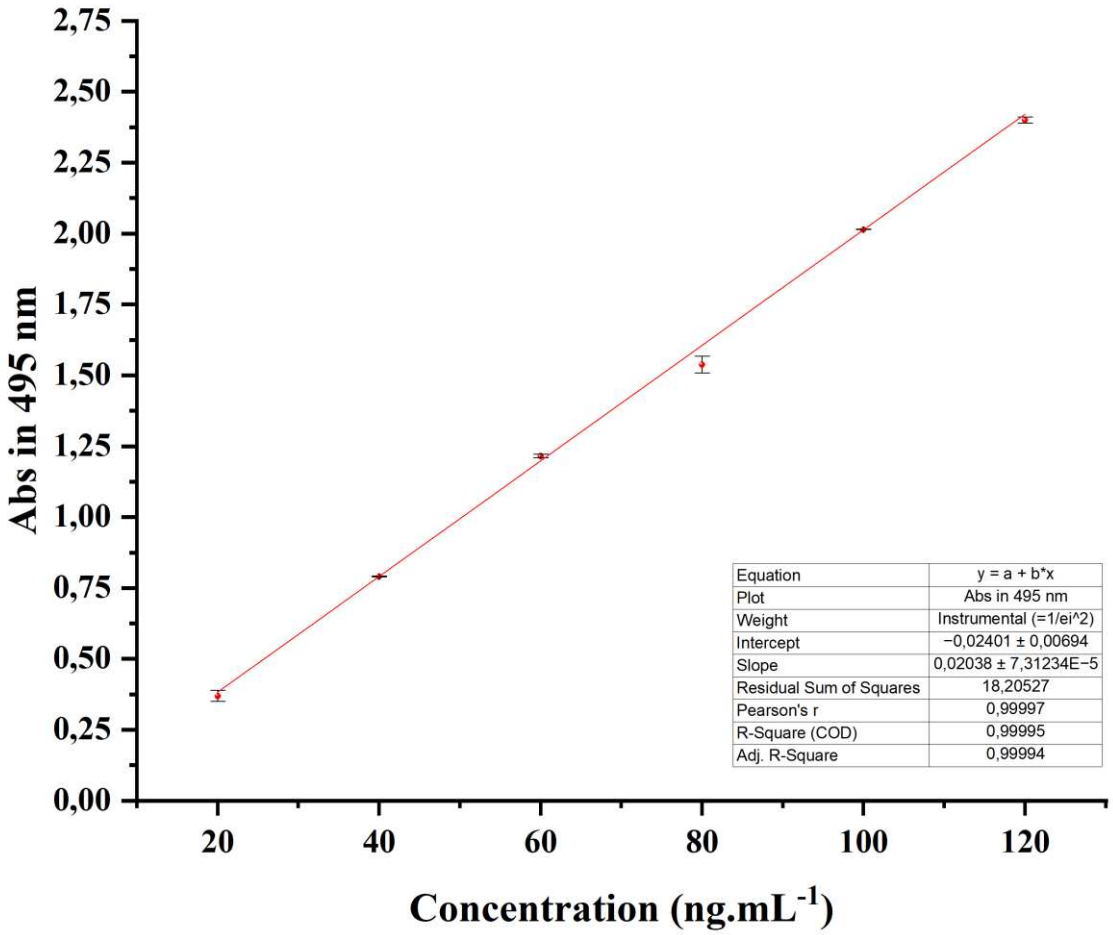

Figure S12 - Composition of biogas in acidogenic reactor (AR) and complete digestion (CAD) reactors.

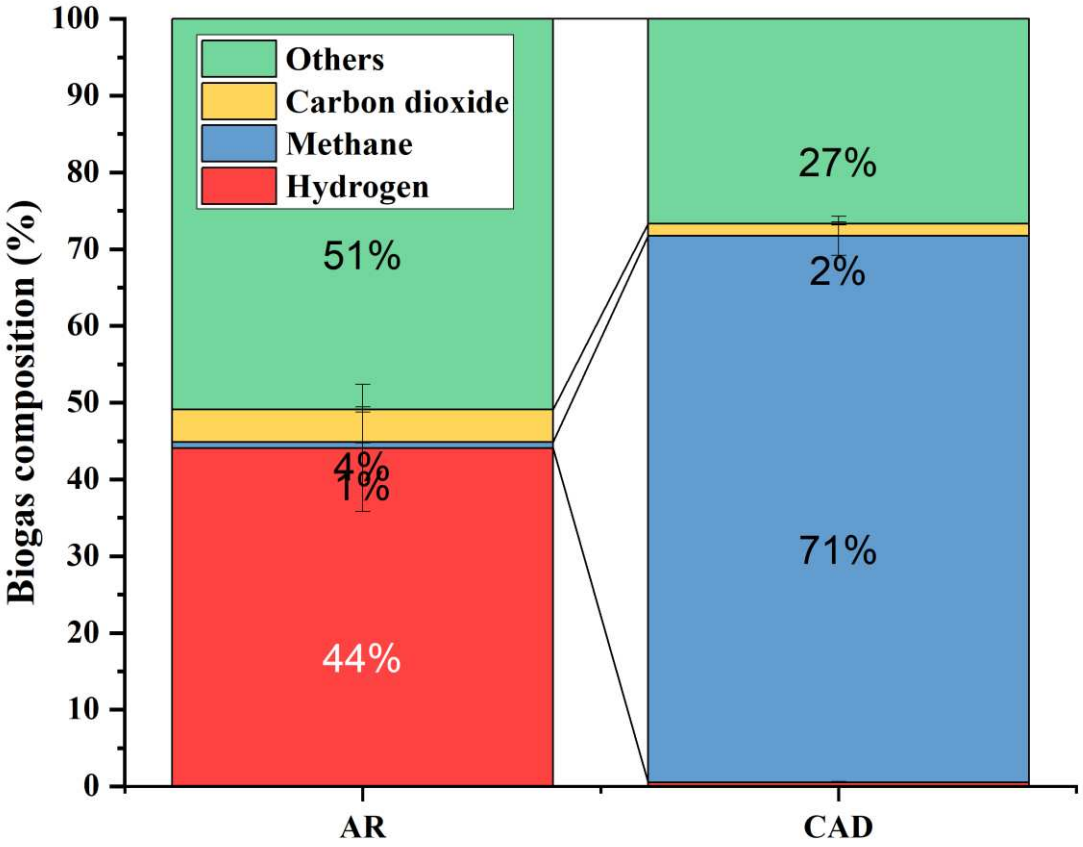

Figure S13 - Phylum proportion at start (t_0) and end (t_f) time.

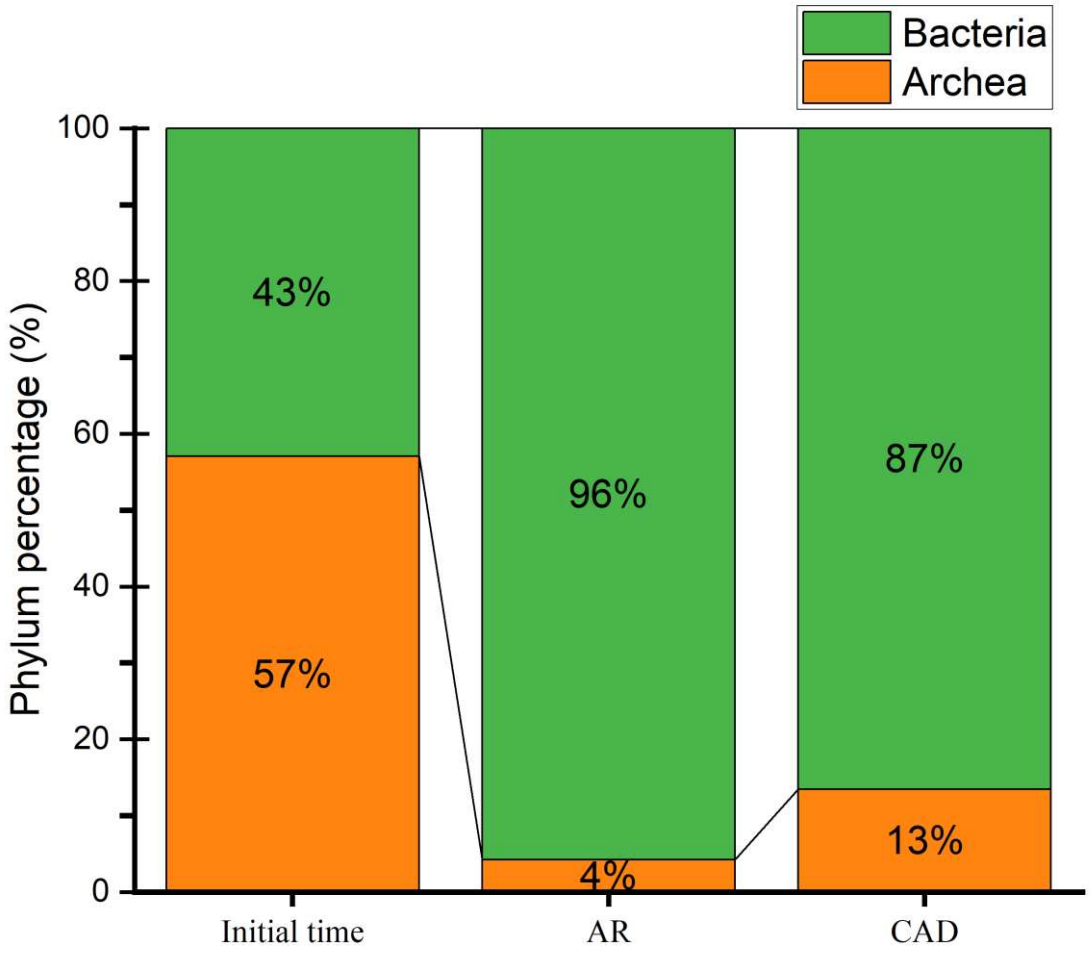

Table S6 - Relative abundance in the intial and final time of assay.

Sample name	**Initial Time (t_0)**		**AR (t_f)**		**CAD (t_f)**	
Total Sum	46090		78965		83439	
Clostridium butyricum	2	0%	16866	21%	10561	13%
Romboutsia lituseburensis	1	0%	11831	15%	8112	10%
Clostridium quinii	0	0%	4443	6%	14378	17%
Methanolinea sp.	14070	31%	495	1%	517	1%
Romboutsia sp. LA1	0	0%	7727	10%	5095	6%
Parabacteroides chartae	1	0%	5233	7%	7159	9%
Methanothrix sp.	8920	19%	801	1%	1417	2%
Thermoanaerobacterales bacterium	6085	13%	1472	2%	3452	4%

Macellibacteroides fermentans	1	0%	2819	4%	4770	6%
Rikenellaceae bacterium	0	0%	7086	9%	291	0%
Methanosarcina mazei	4	0%	6	0%	5012	6%
Terrisporobacter glycolicus	0	0%	3527	4%	1270	2%
Syntrophobacteraceae bacterium	2962	6%	479	1%	935	1%
Methanobacterium sp.	766	2%	1145	1%	1726	2%
Methanothrix soehngenii	1636	4%	551	1%	934	1%
Pelobacter sp.	1315	3%	671	1%	1102	1%
Paraclostridium bifermentans	0	0%	1675	2%	1394	2%
Exiguobacterium aurantiacum	0	0%	1036	1%	1775	2%
Balneolales bacterium	1851	4%	537	1%	347	0%
Bacteria bacterium	430	1%	663	1%	1581	2%
Chloroflexi bacterium	1655	4%	2	0%	1007	1%
Marinilabiliaceae bacterium	1274	3%	338	0%	714	1%
Clostridium peptidivorans	1	0%	306	0%	1900	2%
Clostridium sartagoforme	1	0%	1350	2%	785	1%
Anaerolineaceae bacterium	1025	2%	158	0%	612	1%
Anaerosalibacter sp.	0	0%	1758	2%	0	0%
Caloramator sp.	0	0%	1428	2%	81	0%
Sulfuricurvum kujiense	1125	2%	12	0%	5	0%
Clostridium maritimum	1	0%	267	0%	827	1%
Methanobacterium beijingense	548	1%	230	0%	287	0%
Methanosarcina barkeri	1	0%	1	0%	1055	1%
Longilinea arvoryzae	160	0%	267	0%	572	1%
Pseudomonas resinovorans	881	2%	27	0%	19	0%
Sedimentibacter saalensis	0	0%	168	0%	641	1%
Mesotoga infera	282	1%	142	0%	147	0%
Methanosaeta harundinacea	285	1%	58	0%	98	0%
Clostridium intestinale	0	0%	349	0%	39	0%
Clostridium sp.	0	0%	89	0%	298	0%
Cellulosilyticum lentocellum	0	0%	358	0%	17	0%
Clostridium beijerinckii	1	0%	6	0%	351	0%
Clostridium subterminale	0	0%	221	0%	79	0%
Romboutsia sedimentorum	0	0%	154	0%	145	0%
Terrisporobacter petrolearius	0	0%	180	0%	111	0%

Exiguobacterium aestuarii	0	0%	60	0%	210	0%
Syntrophus gentianae	259	1%	0	0%	6	0%
Oscillibacter ruminantium	0	0%	258	0%	1	0%
Aminivibrio pyruvatiphilus	156	0%	12	0%	72	0%
Clostridium amylolyticum	0	0%	123	0%	108	0%
Clostridium chauvoei	0	0%	83	0%	146	0%
Enterobacter cloacae complex	0	0%	109	0%	82	0%
Methanobacterium petrolearium	30	0%	34	0%	117	0%
Anaerosporobacter mobilis	0	0%	144	0%	27	0%
Bacteroides graminisolvens	0	0%	131	0%	10	0%
Clostridium malenominatum	1	0%	136	0%	3	0%
Clostridium swellfunianum	0	0%	28	0%	106	0%
Clostridium thermopalmarium	0	0%	69	0%	56	0%
Petrimonas sulfuriphila	3	0%	92	0%	24	0%
Thauera phenylacetica	40	0%	15	0%	47	0%
Paraclostridium benzoelyticum	0	0%	37	0%	62	0%
Enterobacter sp.	0	0%	68	0%	26	0%
Clostridium sulfidigenes	0	0%	57	0%	31	0%
Peptoclostridium acidaminophilum	2	0%	2	0%	81	0%
Leuconostoc mesenteroides	0	0%	5	0%	68	0%
Clostridium tertium	0	0%	48	0%	20	0%
Acetoanaerobium noterae	14	0%	18	0%	35	0%
Leuconostoc fallax	0	0%	4	0%	62	0%
Shewanella putrefaciens	57	0%	2	0%	0	0%
Syntrophus buswellii	42	0%	0	0%	15	0%
Proteiniclasticum ruminis	0	0%	18	0%	38	0%
Clostridium paraputrificum	0	0%	51	0%	2	0%
Bacteroides ovatus	0	0%	49	0%	2	0%
Smithella propionica	22	0%	4	0%	12	0%
Bacteroides xylanisolvens	0	0%	30	0%	2	0%
Arcobacter defluvii	31	0%	0	0%	0	0%
Citrobacter murliniae	0	0%	21	0%	9	0%
Lentimicrobium saccharophilum	13	0%	0	0%	15	0%
Weissella confusa	0	0%	3	0%	25	0%

Bacteroides thetaiotaomicron	0	0%	27	0%	0	0%
Anaerotignum propionicum	0	0%	25	0%	1	0%
Streptococcus equinus	0	0%	16	0%	10	0%
Clostridium aurantibutyricum	0	0%	4	0%	21	0%
Methanomethylovorans hollandica	4	0%	6	0%	12	0%
Citrobacter freundii	0	0%	14	0%	7	0%
Clostridium cadaveris	0	0%	21	0%	0	0%
Bacillus bataviensis	0	0%	20	0%	0	0%
Clostridium saccharobutylicum	0	0%	0	0%	20	0%
Enterobacteriaceae bacterium	0	0%	14	0%	6	0%
Methanobacterium kanagiense	6	0%	4	0%	9	0%
Syntrophus aciditrophicus	3	0%	2	0%	14	0%
Methanosarcina siciliae	0	0%	0	0%	17	0%
Rhizobium aggregatum	13	0%	1	0%	3	0%
Anaerobium acetethylicum	0	0%	0	0%	16	0%
Dysgonomonas oryzarvi	0	0%	12	0%	4	0%
Leuconostoc pseudomesenteroides	0	0%	1	0%	15	0%
Desulfuromonas acetexigens	15	0%	0	0%	0	0%
Methanobacterium formicicum	4	0%	1	0%	10	0%
Clostridium tunisiense	0	0%	5	0%	9	0%
Clostridium viride	0	0%	5	0%	9	0%
Desulfovibrio alcoholivorans	11	0%	2	0%	0	0%
Desulfovibrio aminophilus	10	0%	2	0%	1	0%
Enterococcus sp.	0	0%	10	0%	3	0%
Clostridium saccharolyticum	0	0%	12	0%	0	0%
Clostridium disporicum	0	0%	7	0%	4	0%
Clostridium kogasense	0	0%	8	0%	3	0%
Levilinea saccharolytica	4	0%	0	0%	7	0%
Methanosarcina soligelidi	0	0%	0	0%	11	0%
Pseudomonas nitroreducens	0	0%	9	0%	2	0%
Romboutsia timonensis	2	0%	4	0%	5	0%
Clostridium celerecrescens	0	0%	7	0%	3	0%
Seramator thermalis	0	0%	9	0%	1	0%

Trichococcus ilyis	0	0%	4	0%	6	0%
Brachymonas denitrificans	1	0%	2	0%	6	0%
Methanocorpusculum sinense	8	0%	0	0%	1	0%
Pseudomonas stutzeri	9	0%	0	0%	0	0%
Bacillus cereus group	1	0%	3	0%	4	0%
Clostridium puniceum	0	0%	0	0%	8	0%
Desnuesiella massiliensis	0	0%	5	0%	3	0%
Desulfovibrio vulgaris	0	0%	8	0%	0	0%
Exiguobacterium himgiriensis	0	0%	0	0%	8	0%
Hydrogenophaga flava	8	0%	0	0%	0	0%
Leuconostoc lactis	0	0%	2	0%	6	0%
Lactobacillus casei	0	0%	4	0%	3	0%
Sporacetigenium mesophilum	0	0%	5	0%	2	0%
Acinetobacter seohaensis	6	0%	0	0%	0	0%
Enterobacter ludwigii	0	0%	3	0%	3	0%
Flexilinea flocculi	6	0%	0	0%	0	0%
Geobacter lovleyi	6	0%	0	0%	0	0%
Lachnoclostridium phytofermentans	0	0%	6	0%	0	0%
Ornatilinea apprima	2	0%	0	0%	4	0%
Oscillibacter valericigenes	0	0%	6	0%	0	0%
Sinanaerobacter chloroacetimidivorans	0	0%	0	0%	6	0%
Thauera humireducens	0	0%	3	0%	3	0%
Anaerocolumna cellulosilytica	0	0%	5	0%	0	0%
Azonexus hydrophilus	5	0%	0	0%	0	0%
Citrobacter sp.	0	0%	4	0%	1	0%
Enterococcus italicus	0	0%	2	0%	3	0%
Ilyobacter delafieldii	0	0%	3	0%	2	0%
Kluyvera ascorbata	0	0%	4	0%	1	0%
Kluyvera intermedia	0	0%	4	0%	1	0%
Methanocorpusculum labreanum	5	0%	0	0%	0	0%
Syntrophobacter fumaroxidans	2	0%	0	0%	3	0%
Syntrophus sp.	4	0%	0	0%	1	0%
Wolinella succinogenes	1	0%	1	0%	3	0%