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Valorisation biologique des gaz résiduaires de l'industrie sidérurgique : Étude de la biométhanation des syngaz dans des réacteurs à lit ruisselant sous différents paramètres opératoires

Par

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ABSTRACT

The steel industry is a major source of greenhouse gas emissions, largely due to its emission of off-gases. This thesis investigates the potential for bio-upgrading these off-gases through syngas biomethanation in trickle bed reactors (TBRs) by examining the effects of critical operational parameters such as internal packing, pressure, and temperature. The project is systematically divided into three stages. In the first stage, the impact of reactor biocarriers on reactor performance and biofilm formation is evaluated. The second stage assesses the effects of pressure variations on methane yield and process stability. In the third stage, the influence of temperature conditions on microbial activity and overall reactor efficiency is examined. Complementary metagenomic analyses and activity tests are conducted throughout each phase to monitor and elucidate microbial community dynamics.

Results indicate that using a biocarrier with higher surface area resulted in higher volumetric methane production despite slightly lower per-unit activity. Results demonstrate that the key determinant for enhanced reactor performance is the amount of biofilm generated on the biocarriers, with effective surface area emerging as the primary criterion for biocarrier selection. Increasing the reactor pressure up to 2.5 atm during CO-rich syngas biomethanation in TBR did not significantly affect the microbial community structure or volumetric methane production, demonstrating the system's robustness under these conditions. However, due to potential negative effects on H_2/CO_2-based processes as well as increased operational costs and safety concerns, lower pressure was preferred to have an optimize performance while mitigating risks. Thermophilic operation promoted a broader range of thermophilic microorganisms and enhanced substrate uptake, resulting in increased methane production rates; however, this was accompanied by a slight increase in volatile fatty acid accumulation. The findings provide critical insights for optimizing TBR systems to sustainably convert steel industry off-gases into renewable methane, thereby contributing to carbon emission reduction efforts.

Keywords:

Steel industry off-gases, Syngas biomethanation, Trickle-bed reactor (TBR), Biocarrier, Pressure, Temperature

RÉSUMÉ

L'industrie sidérurgique est une source majeure d'émissions de gaz à effet de serre, principalement en raison des gaz résiduaires qu'elle rejette. Cette thèse étudie le potentiel de valorisation biologique de ces gaz résiduaires par biométhanisation de syngaz dans des réacteurs à lit ruisselant (TBRs), en examinant les effets de paramètres opérationnels critiques tels que le type de supports utilisés pour le remplissage interne, la pression et la température. Le projet est systématiquement divisé en trois étapes. Pour la première étape, l'impact des composants internes du réacteur sur ses performances et sur la formation de biofilm est évalué. La deuxième étape examine les effets des variations de pression sur le rendement en méthane et la stabilité du processus. La troisième étape examine l'influence des conditions de température sur l'activité microbienne et l'efficacité globale du réacteur est analysée. Des analyses métagénomiques complémentaires et des tests d'activité sont réalisés tout au long de chaque phase pour observer la dynamique des communautés microbiennes.

Les résultats indiquent que l'utilisation d'un support offrant une surface plus élevée conduit à une production volumétrique de méthane accrue, malgré une activité par unité légèrement inférieure. Les résultats montrent également que la quantité de biofilm formée sur les supports constitue le facteur déterminant de l'amélioration des performances du réacteur, la surface effective disponible étant ainsi le critère principal à considérer pour le choix des supports. L'augmentation de la pression du réacteur jusqu'à 2,5 atm lors de la biométhanisation de syngaz riche en CO dans le TBR n'a pas significativement affecté de la communauté microbienne ni la production volumétrique de méthane, ce qui atteste de la robustesse du système dans ces conditions. Cependant, en raison des effets potentiellement négatifs sur les processus basés sur H_2/CO_2, ainsi que de l'augmentation des coûts opérationnels et des préoccupations en matière de sécurité, une pression plus basse a été privilégiée afin d'optimiser les performances tout en atténuant les risques. L'exploitation en conditions thermophiles a favorisé une plus grande diversité de micro-organismes thermophiles et une meilleure absorption des substrats, entraînant ainsi une augmentation des taux de production de méthane ; toutefois, cela s'est accompagné d'une légère augmentation de l'accumulation d'acides gras volatils.

Ces résultats fournissent des informations essentielles pour l'optimisation des systèmes utilisant des TBRs en vue de convertir de manière durable les gaz résiduaires de l'industrie sidérurgique en méthane renouvelable, contribuant ainsi aux efforts de réduction des émissions de carbone.

Mots-clés :

Gaz résiduaires de l'industrie sidérurgique, Biométhanisation de syngaz, Réacteur à lit ruisselant (TBR), Support, Pression, Température

Résumé de la recherche en français

Introduction

L'augmentation de la demande mondiale d'énergie, due à la croissance démographique et à l'expansion industrielle, nécessite la transition de l'utilisation des sources de combustibles fossiles vers des alternatives durables. L'industrie sidérurgique, un contributeur majeur aux émissions de gaz à effet de serre, génère des gaz résiduaires riches en carbone composés principalement de CO, CO_2 et aussi H_2. Ces gaz présentent une opportunité de valorisation biologique par fermentation microbienne, permettant la production de gaz naturel renouvelable (renewable national gas; RNG) via la biométhanation, ou encore de carburant enrichi en H_2 par la réaction du gaz à l'eau (water-gas shift reaction; WGSR).

La biométhanation du syngaz, un processus biologique utilisant des consortiums microbiens pour convertir ces gaz en méthane, est une approche prometteuse pour réduire les émissions et améliorer l'efficacité des ressources. Cette étude se concentre sur l'optimisation de la biométhanation du syngaz dans des TBRs en étudiant les effets des matériaux de garnissage, de la pression et de la température sur l'activité microbienne, le transfert de masse gaz-liquide et le rendement en méthane. Une meilleure efficacité de conversion et une productivité volumétrique accrue faciliteront l'adoption industrielle, soutenant la circularité du carbone dans la production sidérurgique tout en contribuant à la décarbonisation et aux objectifs de neutralité carbone.

Objectifs de l'Étude

Dans la production sidérurgique intégrée, les gaz résiduaires tels que le gaz de haut fourneau (blast furnace gas; BFG), le gaz de cokerie (coke oven gas; COG) et le gaz de convertisseur à oxygène (basic oxygen furnace gas; BOFG) contribuent de manière significative aux émissions de CO_2. Ces flux de gaz contiennent des agents réducteurs précieux (CO, CH_4 et H_2) qui peuvent être utilisés pour la réduction directe du minerai de fer ou la production d'énergie. Diverses stratégies de valorisation ont été explorées, notamment la récupération d'énergie, la purification des gaz de grande valeur (CH_4 et H_2) ou la conversion en produits chimiques et carburants.

Cette étude se concentre sur la valorisation biologique des gaz résiduaires sidérurgiques via la WGSR et la biométhanation pour produire du RNG. Ces processus biologiques offrent des avantages par rapport à leurs équivalents thermochimiques, tels que l'utilisation de biocatalyseurs économiques, le fonctionnement dans des conditions de pressions et de températures plus douces, une haute tolérance aux impuretés du syngaz et une sélectivité supérieure des produits formés. Bien que la biométhanation utilisant des consortiums microbiens mixtes soit plus étudiée, les souches pures

thermophiles démontrent un potentiel intéressant pour une efficacité supérieure, toutefois à un niveau de maturité technologique inférieur. Mais quoiqu'il en soit, toutes ces approches biologiques minimisent les besoins en produits chimiques et en énergie, réduisent les coûts de prétraitement des gaz et améliorent l'empreinte carbone des procédés en intégrant plusieurs flux de gaz avec une haute sélectivité et un rendement élevé.

Cette recherche vise à optimiser la biométhanation du syngaz dans des TBRs. Elle comporte trois phases : (1) évaluation du remplissage des réacteurs, (2) évaluation des variations de pression et (3) examen des effets de la température, avec des analyses métagénomiques et des tests d'activité pour surveiller les performances microbiennes. Les résultats soutiendront l'intégration des technologies de valorisation biologique des gaz dans la fabrication de l'acier, contribuant à la circularité du carbone et aux efforts de décarbonisation.

Questions de Recherche et Hypothèses

Ce programme de recherche se concentre sur la conversion des gaz résiduaires riches en CO, H_2 et CO_2 de l'industrie sidérurgique en produits à faible teneur en carbone. Les principaux objectifs sont de développer, valider et optimiser des technologies pour la production d'énergie renouvelable tout en réduisant les émissions de carbone. Ultimement, l'étude vise à intégrer le processus de fermentation dans l'infrastructure existante des aciéries pour minimiser les coûts en tirant parti des services et des installations disponibles. La recherche examine les facteurs clés influençant la biométhanation des syngaz dans les TBRs, en particulier :

L'effet du remplissage interne du réacteur – L'étude émet l'hypothèse que les variations des matériaux de garnissage internes affecteront les interactions gaz-liquide, l'activité microbienne et le rendement en méthane. L'optimisation du remplissage interne du réacteur devrait améliorer le transfert de masse et créer des conditions favorables à la croissance microbienne.

L'impact de la pression – Il est proposé qu'une augmentation de la pression de fonctionnement améliorera la production de méthane en augmentant la solubilité des gaz, augmentant ainsi la disponibilité du substrat pour les micro-organismes méthanogènes.

Le rôle de la température – L'hypothèse suggère que les conditions thermophiles amélioreront les taux de réaction et le rendement en méthane en raison d'une activité métabolique microbienne plus élevée. Cependant, la stabilité microbienne à des températures élevées reste une considération clé.

En testant systématiquement ces hypothèses, la recherche vise à approfondir la compréhension de la conception des réacteurs et des conditions de fonctionnement dans la valorisation biologique des gaz,

contribuant finalement à l'optimisation des systèmes de biométhanation à l'échelle industrielle pour la production d'énergie durable.

Matériels et Méthodes

La configuration expérimentale de cette étude a été conçue pour étudier la biométhanation du syngaz dans des TBRs. Le système de bioréacteurs a été conçu avec une configuration à contre-courant triphasée, chaque réacteur étant rempli d'un garnissage interne spécifique. Les supports pour biofilm étaient divisés en trois zones séparées pour optimiser l'activité microbienne et la formation du biofilm. Pour maintenir un contrôle précis de la température, les réacteurs possédaient une double enveloppe, où de l'eau régulée en température circulait. Une sonde de température située au bas du réacteur surveillait en continu les conditions internes, garantissant un fonctionnement stable. De plus, un manomètre en haut fournissait des lectures de pression en temps réel pour maintenir des performances sûres et optimales.

Le système d'alimentation en gaz utilisait des cylindres de gaz individuels pour générer un mélange de syngaz synthétique avec une composition contrôlée. Des débitmètres massiques régulaient le débit de chaque composant gazeux, assurant une livraison précise et cohérente. Les gaz mélangés étaient ensuite injectés par le bas, favorisant un mélange complet et une interaction efficace avec les consortiums microbiens. Les expériences ont été menées dans des conditions mésophiles (36°C) en utilisant des boues granulaires anaérobies provenant d'un digesteur à lit de boue fluidisé (upflow anaerobic sludge blanket; UASB) à grande échelle traitant les eaux usées de transformation de fruits (Lassonde Inc., Rougemont, QC, Canada). Pour les conditions thermophiles (56°C), les boues anaérobies liquides provenaient d'ADM (Archer Daniel Midland, Candiac, QC, Canada).

Les milieux liquides utilisés dans le processus de biométhanation ont été soigneusement formulés pour fournir les macro- et micro-nutriments essentiels, garantissant une croissance et une activité microbienne soutenues. Les milieux soutenaient les voies métaboliques des méthanogènes hydrogénotrophes et les carboxydotrophes, ainsi que celles impliquant la consommation et la production d'acétate. Les composants clés comprenaient l'azote, le phosphore, le potassium et des oligo-éléments essentiels comme le fer, le zinc et le magnésium. Un tampon phosphate (pH 7,5) a été ajouté pour maintenir un pH stable, empêchant l'acidification ou l'alcalinisation qui pourraient perturber le métabolisme microbien, en particulier la méthanogenèse. Chaque réacteur a été initialement rempli avec 5L de milieu liquide, et le temps de rétention hydraulique a été fixé à 28 jours. Pour maintenir l'activité microbienne, une partie du milieu a été remplacée manuellement deux fois par semaine par des nutriments frais, assurant la stabilité du système tout au long des expériences.

Le réacteur comprend un système de support interne conçu sur mesure qui supporte les matériaux de garnissage. Ce support permet une division verticale du TBR en trois zones actives, permettant une analyse microbienne ciblée dans différentes sections au fil du temps. Cette conception permet un échantillonnage spécifique à chaque zone, fournissant des informations sur l'évolution et l'adaptation de la communauté microbienne pendant le processus de biométhanation. De plus, une buse en haut assure une distribution uniforme du liquide, optimisant le contact avec le garnissage interne pour une efficacité de processus améliorée.

Dans la première phase expérimentale, l'effet du modèle de garnissage interne sur l'efficacité de la biométhanation du syngaz a été évalué. Deux types de garnissage ont été testés : le garnissage aléatoire biologique RFK dans le réacteur 1 (R1) et le garnissage en anneau Hiflow (HF) dans le réacteur 2 (R2), chacun avec un volume de travail égal de 18 L. Les deux réacteurs ont été inoculés avec le même consortium microbien (Lassonde broyé) pour assurer la cohérence, avec une concentration initiale de VSS de 5 g/Lpbd (gramme par litre de lit de garnissage par jour; gram per liter of packing bed per day). Les supports de garnissage ont été prémélangés avec l'inoculum avant d'être répartis dans les deux réacteurs. Ensuite, les réacteurs ont été opérés à 36°C à 1,5 atm (au bas) pour favoriser l'enrichissement microbien. Cette phase a évalué comment la surface et les propriétés des matériaux influençaient les performances de la biométhanation. Les réacteurs ont été maintenus dans ces conditions pendant plusieurs semaines pour permettre le développement des biofilms, après quoi le débit d'entrée du syngaz a été progressivement augmenté jusqu'à atteindre un état stable à chaque débit. L'expérience s'est poursuivie pendant quatre mois, testant diverses compositions de syngaz, avec une surveillance régulière pour évaluer les performances et la stabilité sous des débits croissants, identifiant les conditions de fonctionnement optimales.

Dans la deuxième phase expérimentale, qui s'est concentrée sur la variation de la pression, les réacteurs ont été ouverts pour analyse après les quatre premiers mois de fonctionnement, et le type de garnissage le plus performant a été sélectionné pour poursuivre. Les réacteurs ont ensuite été opérés sous deux pressions différentes : 1,5 atm pour le R1 et 2,5 atm pour le R2, la limite supérieure étant choisie en fonction des critères de sécurité du réacteur. Pour maintenir l'activité microbienne, les supports de garnissage couverts de biofilm ont été collectés, mélangés avec des boues fraîches et des supports non inoculés pour reconstituer la moitié manquante, et recréer une concentration initiale de VSS (5,1 g/Lpbd). Ils ont été répartis dans les deux réacteurs. Les réacteurs ont de nouveau été exploités pendant plusieurs mois avec différentes compositions d'alimentation pour évaluer l'effet de la pression sur l'efficacité de la biométhanation. Les réacteurs ont d'abord été maintenus avec des débits de substrats moyens pour permettre la stabilisation des biofilms, suivie d'une augmentation progressive des débits d'entrés de syngaz. Pour chaque débit, la surveillance des performances s'est

poursuivie jusqu'à ce qu'un taux de production de méthane stable soit atteint, garantissant un fonctionnement optimal du réacteur sous chaque condition de pression.

Dans la troisième phase expérimentale, axée sur la variation de la température, les réacteurs ont été réinoculés pour évaluer les performances de la biométhanation dans des conditions mésophiles (36°C, R1) et thermophiles (56°C, R2), après l'optimisation de la pression. Pour maintenir la cohérence microbienne, les boues mésophiles et thermophiles ont été mélangées avec les supports recouverts de biofilm de la phase précédente, qui avaient subi le meilleur traitement de pression, en plus de 18L de nouveaux supports, assurant ainsi le total des 36L pour les deux réacteurs. L'inoculum a été préparé en mélangeant des portions égales de VSS provenant de boues mésophiles fraîches (Lassonde broyée) et thermophiles (ADM) pour atteindre un total de 5 g/Lpbd dans les réacteurs. Cette approche a permis une comparaison directe des effets de la température sur la biométhanation dans les conditions de pression précédemment optimisées.

Les structures des communautés microbiennes dans les TBRs ont été surveillées en analysant à la fois le biofilm sur les supports internes et les populations dans le milieu circulant. Des tests d'activité spécifiques ont été réalisés sur les échantillons initiaux (Lassonde broyé et ADM) et finaux (biomasse sur les supports). L'analyse de la composition des gaz a été effectuée pour quantifier la consommation de CO, H_2 et CO_2, et la production de méthane, H_2 et CO_2, fournissant des informations sur l'efficacité de la biométhanation du syngaz. Des échantillons de gaz ont été collectés quotidiennement à l'aide de seringues étanches et analysés par chromatographie en phase gazeuse (GC) pour une quantification précise. Le rendement en méthane a été évalué en calculant la production de méthane par demande chimique en oxygène (chemical oxgnen demand; COD) consommée, la COD servant d'indicateur du substrat microbien disponible.

Résumé des Principaux Résultats

Dans cette étude, nos résultats ont démontré que l'utilisation d'un réacteur à lit ruisselant (TBR) permettait une amélioration significative de la rétention de la biomasse et de la formation de biofilm, contribuant ainsi à une performance accrue du processus de biométhanation du syngaz. Contrairement aux systèmes de type croissance libre, où le lessivage de la biomasse est un défi, la configuration TBR a favorisé le développement d'un biofilm dense et stable sur les supports internes, comme en témoigne l'accumulation élevée de solides en suspension volatils (VSS) observée. Cette structure de biofilm a permis une meilleure interaction gaz-liquide et une activité métabolique soutenue, optimisant l'efficacité de conversion du gaz en méthane. Ces résultats confirment la pertinence du choix d’un TBR pour la valorisation biologique des gaz résiduaires industriels.

La génération et la rétention de biofilm, mesurées par les solides en suspension volatils (volatile suspended solids; VSS), sont cruciales pour améliorer l'efficacité volumétrique des TBRs. Les supports de garnissage interne avec des surfaces plus grandes permettent une accumulation accrue de biofilm, compensant une activité de conversion par unité plus faible. Cette rétention améliorée de la biomasse maximise l'utilisation du syngaz, stabilise les communautés microbiennes et améliore le transfert de masse gaz-liquide, garantissant les performances stables du réacteur. Par conséquent, la surface est un critère clé dans la sélection des supports, car une accumulation plus élevée de VSS contribue directement à l'optimisation de la biométhanation du syngaz dans les systèmes utilisant des TBRs.

L'étude a également examiné l'impact de la pression du réacteur sur la biométhanation du syngaz. L'augmentation de la pression jusqu'à 2,5 atm n'a pas significativement affecté la structure de la communauté microbienne ou l'efficacité de la production volumétrique de méthane, indiquant que le système est robuste dans ces deux conditions. Cependant, pour les processus basés sur la consommation de H_2/CO_2, une pression plus élevée a entraîné une réduction du pH, affectant négativement les méthanogènes hydrogénotrophes, nécessitant des ajustements opérationnels.

L'effet de la température a également été étudié, en comparant les conditions thermophiles et mésophiles. Les conditions thermophiles ont significativement amélioré la diversité microbienne et la dégradation des substrats, notamment grâce à l'enrichissement de *Coprothermobacter proteolyticus*. Cette bactérie joue un rôle crucial dans la régulation des biofilms en décomposant la biomasse accumulée, empêchant un épaississement excessif qui pourrait limiter le transfert de masse. Bien que les conditions thermophiles aient entraîné une accumulation accrue d'acides gras volatils (volatile fatty acids; VFA), indiquant un déséquilibre potentiel du processus, elles ont également permis des taux de production de méthane plus élevés par rapport aux conditions mésophiles. En revanche, les conditions mésophiles ont favorisé une communauté microbienne plus stable, mais moins diversifiée, conduisant à une cinétique de dégradation plus lente et à des rendements globaux en méthane plus faibles.

Dans l'ensemble, les résultats soulignent l'importance d'optimiser la conception du réacteur, la pression et les conditions de température pour maximiser la production de méthane dans la biométhanation effectuée avec les TBRs.

Implications pour l'Industrie Sidérurgique et Recommandations pour les Travaux Futurs

Cette étude confirme que les réacteurs à lit ruisselant (TBRs) représentent une solution robuste pour la valorisation biologique des gaz résiduaires de l’industrie sidérurgique. Nos résultats démontrent

qu’une architecture favorisant la formation de biofilms stables, associée à un contrôle précis de la pression et de la température, permet d’optimiser l'efficacité de conversion du syngaz en méthane.

Pour une application industrielle, il est recommandé de privilégier des matériaux de garnissage offrant un compromis optimal entre surface spécifique élevée et accessibilité aux substrats, afin de maximiser l'activité méthanogène sans induire de limitations diffuses. De plus, nos résultats suggèrent qu'une pression modérée (autour de 1,5 atm) est suffisante pour maintenir des rendements élevés tout en minimisant les coûts et les risques liés à l’exploitation à haute pression.

Le fonctionnement en conditions thermophiles, bien qu'ayant permis une augmentation de la production de méthane, devra être soigneusement adapté en milieu industriel en tenant compte des contraintes de gestion de la chaleur et de la stabilité microbienne à long terme.

Enfin, la valorisation industrielle pourrait bénéficier de l'intégration d'une alimentation en H_2 externe, issue d'électrolyse verte ou de coproduits industriels, afin d'augmenter la conversion du CO_2 en méthane et d’améliorer encore le rendement global de recyclage du carbone.

Ces observations ouvrent la voie à plusieurs perspectives de recherche, notamment :

- L'optimisation de la distribution interne du liquide pour améliorer encore le transfert de masse gaz-liquide.
- L'étude de la résilience microbienne face à des compositions de syngaz variables et aux contaminants industriels.
- La validation de la performance du système à une échelle pilote semi-industrielle pour confirmer les résultats obtenus en laboratoire.

La composition des syngaz joue un rôle significatif dans l'efficacité de la production de méthane. L'ajustement des ratios de CO, CO_2 et H_2 optimise l'activité microbienne et la production de biogaz. L'ajout d'H_2 externe - produit par électrolyse ou provenant d'autres industries – peut être effectué afin de convertir davantage de CO_2 en méthane, et ainsi augmenter le recyclage du carbone.

En optimisant le garnissage interne, la pression du réacteur, la température de fonctionnement et les compositions des gaz, les TBRs à base de biofilm présentent une solution évolutive et économique pour réduire les émissions dans l'industrie sidérurgique tout en maximisant la production de biocarburants.

Sur la base des résultats de cette étude, plusieurs recommandations peuvent aider à optimiser l'application des TBRs dans l'industrie sidérurgique et orienter les recherches futures :

Alimentation ciblée en gaz – Bien que l'analyse métagénomique n'ait montré aucune différence microbienne majeure entre les zones du réacteur, de futures études pourraient explorer les composants du mélange gazeux à des points stratégiques à l'intérieur du TBR. Cette approche pourrait améliorer le contrôle des voies microbiennes et l'efficacité des réactions tout au long du réacteur.

Évaluation de l'impact des contaminants – Les effluents de l'industrie sidérurgique contiennent des impuretés susceptibles d'affecter la production de biogaz. Il sera crucial d'étudier l'impact de ces contaminants, tel que le dioxyde de soufre, sur l'activité microbienne et les performances du réacteur pour concevoir des bioréacteurs résilients capables de traiter de véritables flux de gaz industriels.

Considérations de mise à l'échelle – La mise à l'échelle des TBRs pose des défis tels que le maintien d'un contact uniforme gaz-liquide, la prévention de la mauvaise distribution des flux (liquide et gazeux) et la minimisation de la chute de pression. Pour résoudre ces problèmes, des modélisations de dynamique des fluides computationnelle (CFD) et des expériences à l'échelle pilote devront être menées pour optimiser la conception des réacteurs, les débits et les matériaux de garnissage. La mise en œuvre de systèmes de surveillance et de contrôle avancés devra également soutenir une exploitation efficace à grande échelle.

Stratégies de bioaugmentation – La biométhanation du syngaz est souvent limitée par la capacité métabolique des consortiums microbiens natifs. L'introduction de souches microbiennes performantes avec des capacités de conversion des gaz supérieures peut améliorer l'efficacité de la production de méthane, en résolvant les principaux goulets d'étranglement du processus et en améliorant les performances globales du réacteur.

En intégrant ces stratégies, les systèmes de biogaz basés sur les TBRs peuvent être davantage optimisés pour des applications industrielles à grande échelle, contribuant aux efforts de rendre plus durables les activités de l'industrie sidérurgique.

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LIST OF ABBREVIATIONS

Water- gas shift reaction	WGSR
Renewable natural gas	RNG
Trickle bed reactors	TBR
Giga ton	Gt
Greenhouse gases	GHG
Carbon capture, utilization and storage	CCUS
Blast Furnace Gas	BFG
Coke-Oven Gas	COG
Basic Oxygen Furnace Gas	BOFG
Dimethyl carbonate	DMC
Dimethyl ether	DME
Electric arc furnace	EAF
Copper Oxide	CuO
Part per million	PPM
Carbon Monoxide Dehydrogenase	CODH
Acetyl-CoA synthetase	ACS
Continuous stirred-tank reactors	CSTR
Partial pressure of CO	P_{CO}
Volumetric mass transfer coefficient	K_{La}
Henry's law constant	H_i
Upflow anaerobic sludge blanket	UASB
Revolutions per minute	rpm
Polypropylene	PP
Polyethylene	PE
Volatile suspended solids	VSS
Volatile fatty acids	VFA
High-performance liquid chromatography	HPLC
Gas chromatography	GC
Thermal conductivity detector	TCD
Chemical oxygen demand	COD
Personal protective equipment	PPE
Standard cubic centimeter per minute	SCCM
Syntrophic acetate oxidation	SAO
Extracellular polymeric substances	EPS

1. Introduction

Energy needs are increasing worldwide due to humanity's population growth and the accelerated development of industry leading to today's goal of replacing current non-renewable and scarce fossil fuel sources. Therefore, it is necessary to find new alternatives for the production of sustainable energy to mitigate these energy needs. Nowadays, the use of renewable energy sources like biomass or solid waste has become one of the most promising sources for energy production, which at the same time supports the reduction of harmful fossil fuel gas emissions that largely contribute to global warming [1, 2].

The steel industry is a major contributor to global greenhouse gas emissions due to its reliance on fossil fuels and the generation of carbon-rich off-gases during production processes. These off-gases, predominantly composed of carbon monoxide (CO), carbon dioxide (CO_2), and hydrogen (H_2), represent a significant resource for bio-upgrading into valuable products [3]. The microbial fermentation of this off-gas to produce renewable natural gas (RNG) by biomethanation, or H_2 enriched syngas fuel by water gas shift reaction (WGSR) can substantially mitigate CO_2 emissions of this industry, while simultaneously providing energy and reducing agents which can be incorporated directly back in the process, implementing carbon circularity in the process itself [4]. Syngas biomethanation, a biological process that converts these gases into methane using microbial consortia, has emerged as a promising solution to mitigate emissions and enhance resource utilization[1, 5]. This research will allow improving conversion rates and volumetric efficiency of those processes, which will ease their industrial adoption. The proposed technology can deliver great economic returns; efficient and clean methods that exceed the performance of current thermochemical treatment methods. Gas fermentation has potential for carbon capture and conversion with improved performance, in addition to the important benefit of clean energy and reducing agent production [6].

This study focuses on the optimization of syngas biomethanation in trickling bed reactors (TBR), with particular attention to the effects of packing materials, pressure, and temperature. By understanding how these parameters influence microbial activity, gas-liquid mass transfer, and methane yield, the research aims to advance the integration of sustainable practices into the steel industry while contributing to broader efforts toward decarbonization and net-zero goals.

In this introduction, an overview of the research background, motivation, and objectives will be provided, followed by a statement of the research problem and key questions addressed in the study. Additionally, the scope and limitations of the research will be outlined to define the context and focus

of the work. Finally, the structure of the thesis will be briefly presented to guide the reader through the subsequent chapters.

1.1 Background and Motivation

In mining, metallurgical and iron and steel industry, the impact on climate change and how the industry can respond to it has increasingly been a topic of discussion over the past decade. Under the 2015 Paris Agreement, 195 countries pledged to limit global warming to well below 2.0°C, and ideally not more than 1.5°C above pre-industrial levels (figure 1). That target, if pursued, would manifest in decarbonization across the industry, creating major shifts in commodity demand for the metal extraction and processing industry.

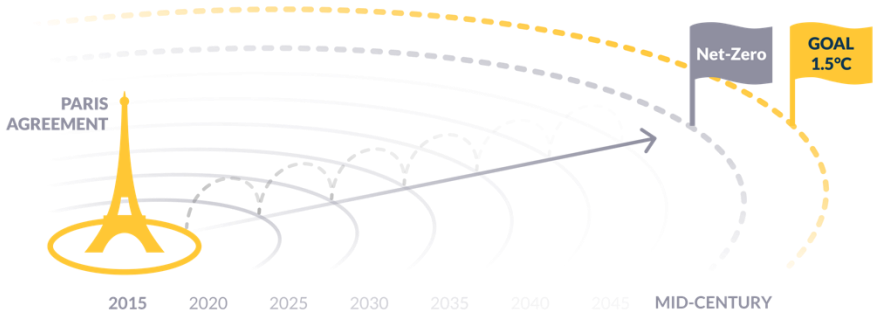


Figure 1) 2015 Paris Agreement target to limit global warming (Data Source: Climate Watch)

Climate change is reshaping the mineral resource investment landscape. Drivers of change – from the affordability of renewable energy to government policies to the world's commitment to reduce CO_2 emissions – have created the perfect environment for the rise of low-carbon technology [7]. The penetration of renewable energy in the energy sector will be crucial to achieve a low-carbon future. Major finance institutions are divesting from thermal coal and investing in the energy transition economy. Combined investments in low-carbon energy technologies have reached 30% of global energy investments. The energy sector today accounts for 41 percent of carbon emissions worldwide, or 13.6 GtCO2e [8], and is expected to rise further as the global population grows, particularly in developing countries, and energy consumption rises because of this new demand.

Canada ranks as the world's 10th largest emitter of greenhouse gases, contributing approximately 1.54% of global emissions. A significant portion of these emissions stems from the energy sector, which accounts for nearly 80% of Canada's total greenhouse gas output (figure 2). This is primarily driven by the nation's reliance on fossil fuels for electricity generation, transportation, and industrial processes, alongside its role as a major producer and exporter of oil and gas. These factors underscore

the critical need for Canada to transition to cleaner energy sources and implement more sustainable practices to reduce its carbon footprint while maintaining economic growth.

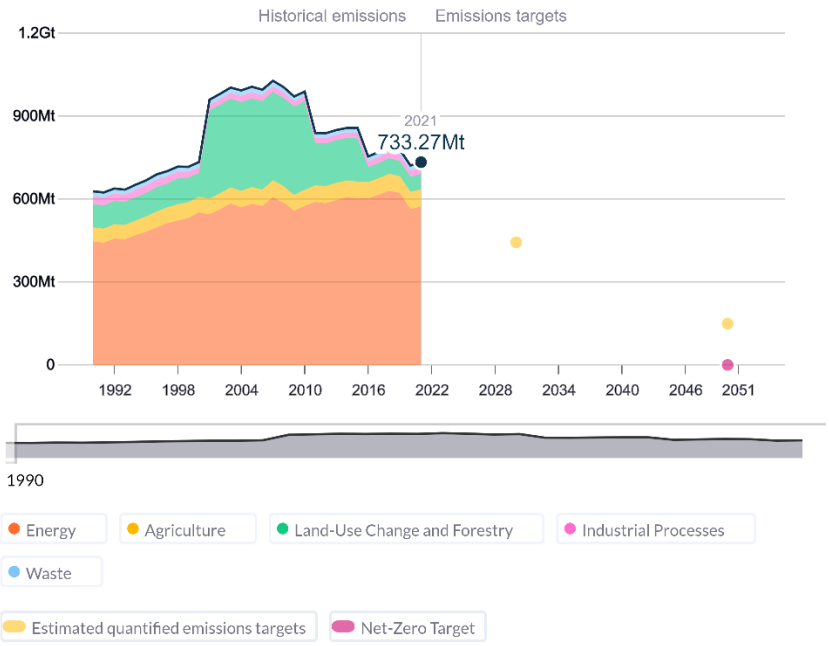


Figure 2) GHG emissions expressed as CO2 equivalent and emission targets in Canada (Data Source: Climate Watch)

1.2 Problem Statement

A core challenge in the energy transition and deep decarbonization is the growing demand for primary energy services. It is widely understood that man-made climate change is chiefly caused by greenhouse gas emissions, especially CO_2, and that the consequences of global warming will be profound, widespread and destructive [(IPCC,2018]]. Nonetheless, global emissions have risen more or less continuously for the past 25 years. This is chiefly due to the growing demand of energy and of products that require energy for their production.

Nowhere is this challenge more evident than in the industrial sector, which has grown profoundly and rapidly over the last 20 years [8]. Steel, a major sector from a commercial and emissions standpoint, is an essential material for the modern world and is a key component of many national economies, with approximately half of the world's steel production occurring in China (figure 3). It is used in construction, military and defense, and manufacturing (e.g., automobiles). A globally traded commodity, iron and steel production has tripled production since 2000, with 2018 seeing $2.5 trillion in sales [9]. It is also an enormous source of greenhouse gases: today's iron and steel industry generate roughly 6% of global CO_2 emissions. Since world steel production is expected to rise in the following years, CO_2 and carbon emissions will increase accordingly if no proper countermeasures are adopted [10].

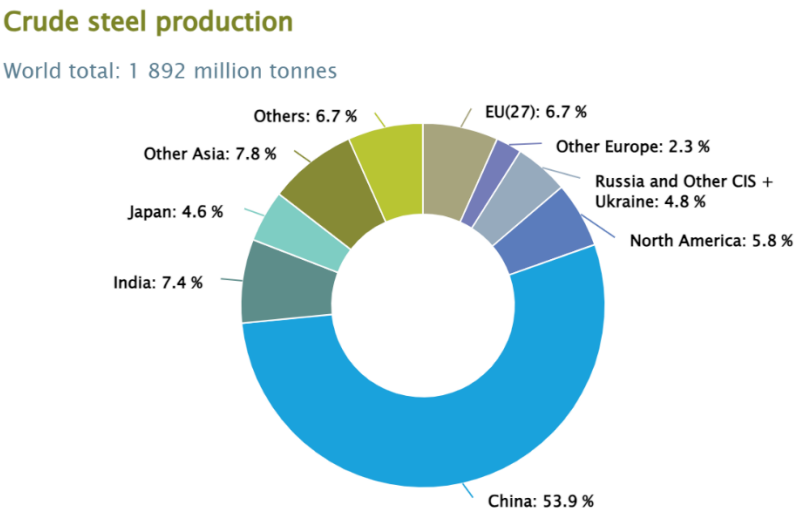


Figure 3) Global distribution of crude steel production in 2023 (data source: Worldsteel)

Capital investments are required to achieve most of the decarbonization potential, but certain measures, such as the adoption of renewable energy, electrification, and operational efficiency, are economic today for many mines. Recent emissions scenarios to limit warming below 2 °C proposed that the CO_2 budget of iron and steel emissions should be capped at 50 Gt between now and 2050. Meeting this budget is challenged by the persistently growing steel demand, which is expected to rise from 1.82 Gt-steel in 2020 to 2.55 Gt-steel in 2050, driven by urbanisation and industrialisation in non-OECD countries (figure 4). To achieve the 2 °C climate goal proposed, the iron and steel sector will need to reduce the emission intensity from 1.58 t of CO_2 per tonne of crude steel in 2020 to a target of 0.52 t of CO_2 per tonne in 2050. In recent decades, the iron and steel industry has reduced its energy intensity by 60%, and most recent iron and steel production sites operate close to a thermodynamic limit of 20 GJ per tonne of crude steel, still leaving an emission gap with respect to the requirement for a sectoral 2 °C target.

The carbon capture, utilisation and storage (CCUS) remains a critical option that could ultimately achieve a deep reduction in CO_2 emissions from this sector, although its costs and externalities remain to be assessed in practical industrial cases for coupling CCUS facilities with steel production. However, the high incremental cost of deploying CCUS is still far from an acceptable level. Furthermore, the net-zero emission steel target in the long term necessitates more breakthrough technologies such as hydrogen- and green electricity-based metallurgy and smart carbon usage (EUROFER 2020).

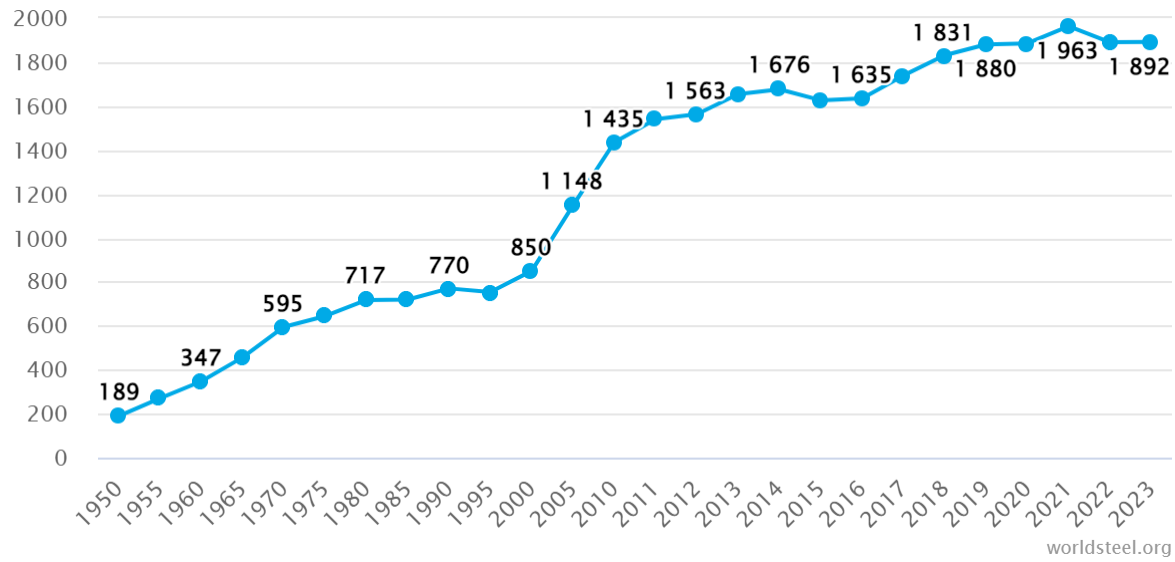


Figure 4) World crude steel production (Mtons) (data source: Worldsteel)

Renewable energy has been one of the largest growing sources of installed electricity generating capacity, growing from 1,058 GW in 2008 to 2,179 GW in 2018 (IRENA 2018), largely driven by government policies, regulations, and incentives as means to decarbonize the energy sector and limit the negative impacts of a changing climate.

Although it is not widely recognized, clean energy technologies such as wind, solar and batteries are actually more material intensive than current traditional fossil-fuel-based energy systems. Meanwhile, demand for minerals and metals such as copper, lithium, cobalt, graphite and nickel – all of which are used in low-carbon technologies - will gather pace in the future and sustainable and reliable extraction and production will need to keep up. A new World Bank Group report, "Minerals for Climate Action: The Mineral Intensity of the Clean Energy Transition", finds that the production of minerals, such as graphite, lithium and cobalt, could increase by nearly 500% by 2050 (rise by 965% for lithium, 585% for cobalt, 383% for graphite, 241% for indium, 173% for vanadium) to meet the growing demand for clean energy technologies. It estimates that over 3 billion tons of minerals and metals will be needed to deploy wind, solar and geothermal power, as well as energy storage, required for achieving a below 2°C future.

At a time when the world is shifting to a low-carbon future and combating climate change, development institutions are accelerating commitments to change the way we source minerals and metals. Adopting biotechnological and microbial processes presents a sustainable alternative to conventional mineral extraction methods. These innovative approaches can enhance efficiency, reduce environmental degradation, and align with the growing demand for eco-friendly solutions in a low-carbon economy.

1.3 Research Questions and Hypotheses

This research project focuses on the conversion of residual CO-rich gases derived from the iron and steel industry and other brownfield applications into low-carbon products. The research questions for this study include:

- How do different reactor internals affect the performance of bio-upgrading steel industry off-gases in TBR?
- What is the impact of pressure variations on methane production during syngas biomethanation?
- How does temperature influence methane production efficiency in TBR-based biomethanation?

The hypotheses for this study are designed to address the critical factors influencing syngas biomethanation in trickle-bed reactors (TBRs):

Effect of Reactor Internals: It is hypothesized that varying the type and configuration of internal packing materials within the bioreactor will influence gas-liquid interactions, microbial activity, and methane yield. By optimizing these internals, the study expects to enhance mass transfer and create favorable conditions for microbial growth.

Impact of Pressure: The hypothesis suggests that increasing the reactor's operating pressure will boost methane production by enhancing gas solubility in the liquid phase, which in turn improves substrate availability for methanogenic microorganisms.

Role of Temperature: It is proposed that operating under thermophilic conditions will increase reaction rates and methane yields compared to mesophilic conditions due to improved microbial metabolic activity at higher temperatures. However, maintaining microbial stability under these conditions is also critical.

By testing these hypotheses, the research aims to develop a deeper understanding of the interplay between reactor design, operating conditions, and biological processes, ultimately guiding the optimization of industrial-scale biomethanation systems.

1.4 Objectives of the Study

In the steel production process, considering the integrated primary route (using the technology of the BF), three main off-gases are available and have to be considered for use: the Blast Furnace Gas (BFG), the Coke-Oven Gas (COG) and the Basic Oxygen Furnace Gas (BOFG). These three streams are responsible for a large part of direct CO2 emissions in the steel industry. Each stream exhibits specific

characteristics (flow and compositions). These gases contain a significant amount of reducing agents (CO, CH4 and H2) that can be used for the direct reduction of iron ore or for power generation. During the past few decades, several alternatives to valorize the steelwork off-gases have been proposed, including their use for energy production, for valuable compounds recovery or for chemicals and fuels production.

Steelwork off-gases from integrated steelworks can be utilized in several ways, classified into three main categories: (1) used as fuel within the steel mill or nearby power plants, or as reducing agents in steel production, (2) recovery of valuable compounds like H_2 or CH_4, and (3) integration with the chemical sector to produce high-value products such as methanol, Dimethyl carbonate (DMC), Dimethyl ether (DME), or urea, including processes like CH_4 reforming (from methanation) or WGSR to enhance hydrogen availability. The proposed project focuses on the biological upgrading via those last two processes, which are the biological WGSR and the biomethanation, to obtain RNG.

The biological process presents again several advantages over it, such as the use of inexpensive biocatalysts, milder operational conditions, higher tolerance to the impurities of syngas, and higher product selectivity. Biomethanation can be achieved by microbial mixed population, on which extensive research are ongoing due to its versatility of waste streams that can be used (e.g., syngas from biomass gasification). The use of thermophilic pure strains combinations is also under investigation, promising a higher volumetric and yield efficiency, but this research state is at lower TRL than biomethanation with mixed cultures. Therefore, biological WGSR and biomethanation have minimal chemical and energy requirements, avoid the use of expensive catalysts and have a lower, environmentally sustainable infrastructure footprint. Globally, they decrease the cost of gas pretreatment, reduce the number of process steps required, and have the ability to utilize multiple gas streams feedstocks, at high selectivity and yields.

The objectives of this study are to investigate the potential of bio-upgrading steel industry off-gases using syngas biomethanation in a trickle-bed reactor (TBR). The study will explore the effects of key operative parameters, including reactor internals, pressure, and temperature, on methane production. The project is divided into three stages: (1) evaluating the impact of reactor internals on performance, (2) assessing the effect of pressure variations, and (3) examining the influence of temperature conditions, while also conducting metagenomic analysis and activity tests to monitor microbial activity throughout each phase.

1.5 Structure of the Thesis

This thesis is organized into six chapters to provide a comprehensive understanding of the study. The first chapter introduces the subject, offering an overview of the research topic and its significance.

Second chapter includes a detailed description of the principles underlying the process and a review of relevant literature to establish the study's context. The current limitation for production of biofuels, the main bioreactor designs used so far and the microbiological development and potential in syngas conversion process are also overviewed. Additionally, the key operative parameters investigated—such as reactor internals, pressure, and temperature—are introduced.

The third chapter, Materials and Methods, provides a comprehensive description of the experimental setup and design, including the protocols used to carry out the experiments. It also details the methodologies for metagenomic analysis, microbial activity tests, and the analytical methods employed to measure key parameters and evaluate results. This chapter serves as a critical foundation, outlining the tools, techniques, and processes utilized to address the research objectives and ensure reproducibility and accuracy in the study.

The fourth chapter, Results and Discussion, is dedicated to presenting and analyzing the findings from the experiments, which are divided into three distinct phases. Each phase investigates the effect of a specific operative parameter—reactor internals, pressure, and temperature—on the process. The results include detailed activity tests, metagenomic analysis, and other experimental outcomes. These findings are thoroughly discussed in the context of their implications, highlighting trends, patterns, and their alignment with the research objectives and existing literature. This chapter connects experimental observations with scientific interpretation.

Chapter five, Conclusions and Recommendations, summarizes the key findings from the study, highlighting the effects of different operative parameters on the process. It discusses the implications for the steel industry, particularly in improving sustainability and reducing carbon emissions. The chapter also offers recommendations for future research, suggesting areas such as scaling up the process, and further optimizing experimental conditions for industrial applications. These insights aim to guide the next steps in advancing clean energy solutions for the steel sector.

Chapter six, References, compiles all the scholarly sources and materials cited throughout the thesis.

2. Literature Review

In this chapter, I aim to provide a comprehensive review of the literature related to the bio-upgrading of steel industry off-gases. The discussion will begin by exploring the environmental challenges posed by carbon emissions in the steel industry and the significance of developing sustainable solutions. It will include an overview of existing technologies, with a focus on microbial and biotechnological approaches, to reduce carbon emissions and transform off-gases into valuable products.

Additionally, I will delve into the critical operative parameters that influence these processes, such as reactor design, pressure, temperature, and microbial consortia, emphasizing advancements in syngas biomethanation. By examining recent studies and their findings, this chapter aims to highlight gaps in current knowledge and establish the scientific framework that supports the objectives of this research.

2.1 Steel Industry Off-Gases: Characteristics and Utilization

In Canada, steel is produced at 13 plants in five provinces (Alberta, Saskatchewan, Manitoba, Ontario and Quebec). The industry is concentrated in Ontario, with six plants operating there. Steel plants are divided into two general categories according to their major source of metal. Plants that produce steel from iron ore using the BF and BOF process are referred to as integrated plants. Plants that produce steel by melting steel scrap in the electric arc furnace (EAF) process are referred to as EAF plants. One Canadian integrated plant also uses the EAF process to produce a portion of its steel. All four integrated steel plants are in Ontario. In this context, accurate quantification of energy use and associated carbon emissions becomes essential for assessing the effectiveness of decarbonization strategies within the steel sector.

Canadian industry increasingly sees the need for accurate data on energy use. Publicly available data on energy use for the Iron and Steel industry are collected by Statistics Canada (StatCan) using a variety of surveys that are completed by selected industry respondents and energy distribution agencies. Production data were collected by StatCan until May 2013 and are now obtained from the Worldsteel Association. GHG emissions data are calculated by multiplying the energy use of each fuel by Environment and Climate Change Canada's CO_2 conversion factors as published in their annual National Inventory Report [11]. Correspondent CO_2 emissions to energy consumption factors are resumed in Table 1.

Table 1) CO2 emissions factors (NRCan 2007)

Energy source	Factor	Unit
Carbon	3664	gCO_2/t
Coal	3000	gCO_2/t
Coke	3227	gCO_2/t
Coke Oven Gas	45	gCO_2/GJ
Blast Furnace Gas	280	gCO_2/GJ
Basic Oxygen Furnace Gas	185	gCO_2/GJ
Natural gas	56	gCO_2/GJ
Heavy Oil	3170	gCO_2/t
Light Oil	3170	gCO_2/t
High Pressure Steam	267	gCO_2/t
Medium Pressure Steam	240	gCO_2/t
Low Pressure Steam	224	gCO_2/t
Electricity	856	gCO_2/kWh
Oxygen	556	gCO_2/Nm^3
Nitrogen	171	gCO_2/Nm^3
Compressed air	103	gCO_2/Nm^3
Industrial Water	86	gCO_2/km^3

The steelmaking process involves several steps and can be broadly categorized into two main methods: the primary route and the secondary route[12].

Primary Route: This method involves producing steel from iron ore extracted from mines. The iron ore, which contains about 60% iron, must first be reduced to iron using a reducing agent like coke. The primary route includes three processes: the integrated route (Blast Furnace and Basic Oxygen Furnace), smelting reduction, and direct reduction. The integrated route is the most common, representing a significant portion of global steel production.

Secondary Route: This method involves producing steel from recycled scrap steel. The scrap steel is melted, usually in an electric arc furnace, to produce new steel. This method is more direct as it bypasses the need for reducing iron ore.

The main difference between the two methods is that the recycled steel can be directly melted to produce new steel (usually using an electric arc furnace). Thereby, iron ore, which is a mixture of iron oxide (with an iron content of about 60%), must first be reduced to iron by means of a reducing agent (mainly coke, and much less widespread natural gas or H2). For the primary route, three different

processes have been developed: the integrated route including a Blast Furnace (BF) and a Basic Oxygen Furnace (BOF), the smelting reduction process and the direct reduction process [12].

The major processes of the steel industry (e.g., steelmaking and coking) generate numerous wastes and residual gases on site, along with heat and electricity. During the primary steel production route, carbonaceous off-gases are generated during the main production steps of:

(1) conversion of coal to coke in the coke oven, (2) pig iron production in the blast furnace and (3) processing of pig iron to steel in the basic oxygen furnace [10]. These gases are rich in carbon-containing compounds such as CO, CO_2, and varying levels of H_2 and CH_4. Their composition makes them both a challenge and an opportunity, as they represent a substantial source of greenhouse gas emissions while also holding potential as a feedstock for value-added processes. Since the usage of fossil fuels (usually coal and natural gas) as reducing agents in the blast furnace is intensely optimized, alternative ways are investigated for the reduction in those emissions. Generally, a common way to avoid the flaring of steelmaking off-gases is their use as internal energy sources both for heating and power production. As a consequence, a reduction in natural gas and external produced electricity use are obtained, resulting in a decrease in emissions, primary resource consumption, and operating costs [13].

2.1.1 Composition and Challenges

Table 2 depicts the total volumetric amounts and the mean composition of the steelworks off-gases, for a steel plant producing 6 MT steel per year [12]. According to the available flow rates, the BFG stream is by far the largest stream having a flow rate about 20 times larger than the other two available streams. This fact is important to define how these gases may be mixed in relation to the construction of a global valorization flow sheet.

Table 2) Mean composition of steelworks off-gases [12]

Mean Composition (mol.%)	BFG	COG	BOFG	mix
CO	23.5	4.1	54.0	23.9
Ar + O_2	0.6	0.2	0.7	0.6
H_2	3.7	60.7	3.2	6.5
CO_2	21.6	1.2	20.0	20.5
N_2	46.5	5.8	18.1	43.3
CH_4	0.2	22.0	0.0	1.1
CnHm	0.0	2.0	0.0	1.1
H_2O	4.0	4.0	4.0	4.0
Potential impurities * H_2S, SO_2, Organic sulfur, HCN, NH_3, NO_x, BTX, halogens, heavy metals				
Total amount (M_3/h)	730000	40000	35000	805000
Lower heating value (kJ/Nm_3)	3365	15660	7163	4141
Thermal Power (MW)	682	174	70	926

The first generated gas during the steelmaking process is the COG, produced in the production stage of coke from coal in the coke ovens by pyrolysis (figure 5). Coke is a prime raw material in the production of steel, it may be used as fuel to increase the BF temperature, but also as a reducing agent of iron ore. The COG is the most easily exploitable gas because it is composed of a large part of H_2 that can react with CO_2 and CO to potentially generate a high value-added product. Moreover, the amount of available H_2 can be increased by the reforming of the CH_4 (present in the gas or produced by Sabatier's reactions) or by the CO shift reaction [14].

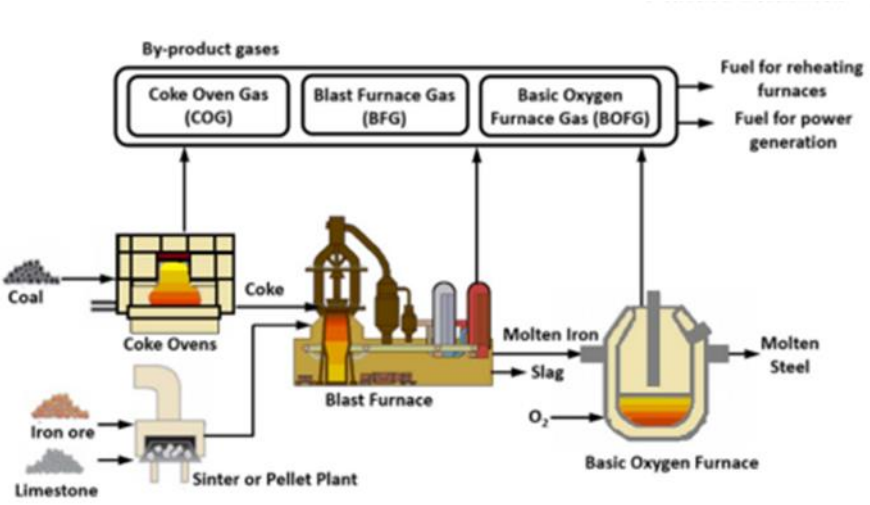


Figure 5) The integrated steelmaking process and the typical utilization of steelwork off-gases [12]

The second gas generated is the BFG corresponding to the output stream of the BF. It contains a large amount of N_2 because air is used as an oxidant. Unfortunately, the amount of available H_2 is small. These facts hinder the direct use of this stream to generate a high value-added product. Other compounds contained in this stream (CO_2 and CO) are generated by the combustion of the coke with oxygen to increase the furnace temperature and to reduce the iron ore. The actual composition of the gas varies and depends strongly on the operating conditions of the blast furnace, such as the levels of the blast oxygen enrichment and the mix of solid reagents employed (coke, pulverized coal, sinter, pellets, ore, etc.).

The third considered gas is the BOFG. It is generated in the BOF, where the molten iron from the BF is introduced. The primary function of the BOF converter is to refine pig iron to steel and this involves desiliconisation, decarburisation, and dephosphorisation of the metal by reaction with high- purify oxygen. Oxygen is injected to burn the carbon in the iron and then to produce molten steel. The major component in this stream is CO. Since CO is a reducing agent, this stream is often used as feed to the BF, in substitution of a part of coke [12].

2.1.2 Current Utilization Methods

In the case of CO_2, three general cases of utilization are possible, utilization without transformation, utilization after chemical transformation, and utilization after biological transformation (Figure 6).

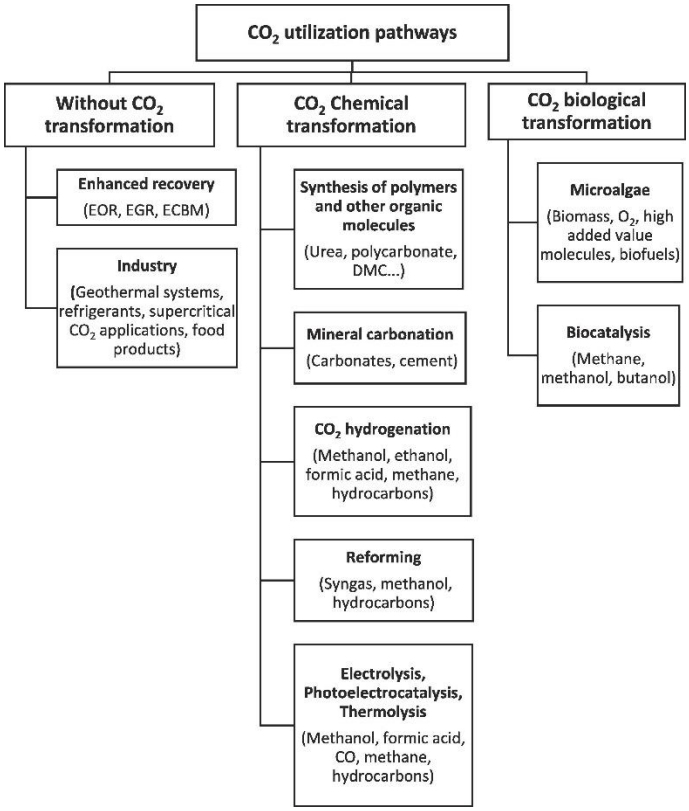


Figure 6) CO2 utilization pathways detailing main utilization processes and resulting products [10]

The CO_2 utilization pathways can be categorized into three main groups: without CO_2 transformation, CO_2 chemical transformation, and CO_2 biological transformation. While chemical transformation includes processes like CO_2 hydrogenation, reforming, and electrolysis to produce fuels and chemicals such as methanol, methane, and hydrocarbons, biological transformation relies on microbial activity and biocatalysis to convert CO_2 into valuable products under milder conditions.

Utilization of steel off-gases can be categorized into three primary approaches: energy recovery, chemical synthesis, and biological conversion:

- Energy Recovery: Off-gases are used as fuel within the steel mill or in nearby power plants, contributing to energy efficiency and reducing the need for external fuel sources [15].
- Chemical Synthesis: The carbon content of off-gases is harnessed to produce valuable chemicals such as methanol, urea, or formate, integrating chemical processes to create high value-added products [16].
- Biological Conversion: Microbial processes convert carbon monoxide-rich exhaust gases into biofuels and chemicals, turning steel mills into biorefineries [17]. Biological conversion plays a significant role within CO2 biological transformation, utilizing microbial processes to convert CO or CO2-rich industrial exhaust gases into biofuels and chemicals. For instance, syngas biomethanation has gained attention due to its sustainability, where syngas (produced from steel mill off-gases) is biologically converted into biomethane. This process effectively transforms steel mills into biorefineries, contributing to renewable energy production while reducing greenhouse gas emissions.

The syngas, which is generated from the steelwork off-gases, is an intermediate that enables creating a broad range of products, such as methane, methanol, synthetic petroleum components via the Fischer–Tropsch process [18] or ammonia and then urea. The above products could then be synthesized from the steelwork off-gases. In this way, COG is used to manufacture ammonia in some fertilizer plants. Steel plants usually have air separation units in order to produce oxygen which is required for steel production, so the N_2 is available from this source[12]. The process involves recovering H_2 from the off-gases and mixing it with N_2 for ammonia synthesis. Urea synthesis also requires CO_2, which can be sourced from BFG.

Most publications consider methanol as the most interesting high value-added products to be synthesized [19]. Indeed, the methanol is a versatile product that is involved in creating a broad range of products, such as formaldehyde, methyl acetate, acetic acid, DME, DMC, olefins, methyl tert-butyl ether (MTBE), ethanol, among others. Some separations or recovery processes are needed to adjust the syngas composition. For example, CO, H_2 and CH_4 recovering processes, as described previously,

are preliminary steps in the methanol synthesis process. Another important separation step is the separation of CO_2 from the BFG and the BOFG if CO_2 conversion is required [20].

The other pathway is water gas shift reaction (WGSR). The WGSR is a thermodynamically equilibrated chemical reaction that consists in the production of CO_2 and H_2 from a mixture of CO and H_2O:

$$CO + H_2O \Leftrightarrow CO + H_2 \quad \Delta H^\circ (298K) = -41.1 \text{ kJ/mol}$$

In this way, an increase in the amount of available H_2 can be carried out. It especially occurs in the Haber-Bosch process for the production of ammonia. Given that the reaction is exothermic, by elevating the temperature, the equilibrium tends to the reactant formation under the principle of Le Châtelier. Thus, it is interesting to develop low temperature processes to increase the conversion of CO. Historically, the thermochemical process took place at a relatively high temperature, in order to have high reaction rates, using an iron catalyst. But as a result of the increased cost of energy, the development of new and more active copper catalysts made possible the development of new processes in two steps [21].

So, at an industrial scale, the WGSR is mainly performed using a cascade of adiabatic reactors with a high temperature step (HTS: High Temperature Shift) followed by a low temperature step (LTS: Low Temperature Shift) with a cooling inter-stage [22]. Thus, the HTS takes advantage of high reaction rates, but is thermodynamically limited, which results in an incomplete conversion of CO with a corresponding content in the output between 2% and 4% (molar fraction). With the decreased temperature in the LTS, the equilibrium is moved towards the production of H2, which enables to obtain a CO content of less than 1% (molar fraction).

2.2 Syngas Biomethanation: Principles and Applications

One of the most promising approaches within second- and third-generation biofuel technologies is the process of gas fermentation, which has gained increasing interest in recent years for the conversion of both industrial off-gases and recalcitrant feedstocks when coupled to their gasification into synthesis gas [23, 24]. Syngas biomethanation refers to the biological conversion of syngas—composed primarily of CO, CO_2, and H_2—into CH_4 by specialized microorganisms. This process leverages the metabolic capabilities of anaerobic microbes, primarily methanogenic archaea, to transform waste gases into renewable energy. It provides an alternative to traditional chemical routes, offering lower energy requirements and a more environmentally friendly approach.

The principle of syngas biomethanation revolves around the anaerobic digestion pathway, where hydrogenotrophic methanogens utilize H_2 and CO_2 to produce methane, while carboxidotrophic

methanogens can convert CO into CH_4. This dual capability allows the utilization of both components in syngas, maximizing conversion efficiency.

Applications of syngas biomethanation span across sectors such as renewable energy production, biogas upgrading, and waste gas valorization, making it a pivotal process in transitioning toward a circular carbon economy. As a potential product, biogas (or biomethane when upgraded) presents a significant potential for its integration into the current biofuel landscape due to its versatility as an energy carrier. To date, the most common practice is to exploit biogas in situ for production of combined heat and power as the quality standards for this application are generally lower. However, biogas upgrading to biomethane provides a more flexible application of this fuel, as biomethane and natural gas are fully miscible in the natural gas grid [1].

Key challenges for the implementation of biomethanation include optimizing reactor conditions, such as pressure, temperature, and pH, and ensuring the stable performance of microbial consortia under varying syngas compositions. Emerging advancements in reactor designs, like trickle-bed reactors (TBRs), enhance gas-liquid mass transfer and microbial efficiency, enabling industrial-scale applications [25].

2.2.1 Microbial Conversion Processes

The conversion of syngas into CH_4 can take place either directly through the conversion of both CO and H_2/CO_2 by carboxydotrophic and hydrogenotrophic methanogenesis. Indirect pathways for CH_4 production involve acetate as an intermediate, such as in homoacetogenesis and carboxydotrophic acetogenesis, or rely on H_2/CO_2 through carboxydotrophic hydrogenogenesis when carbon and energy sources originate from CO. Additionally, acetogenic bacteria have been reported to produce other by-products besides acetate, like ethanol, butyrate, and butanol, which could be further converted into acetate and ultimately into CH_4 [26]. Therefore, a microbial consortium may convert syngas into CH_4 through a complex network of interconnected biochemical reactions as shown in Figure 7. However, the microbial interactions within a stable and structured microbial consortium do not simply consist of cross-feeding relationships, as there are other possible microbial interactions besides cross-feeding, like synergistic interactions between different species and mutual exclusion relationships between metabolically competitive populations [27].

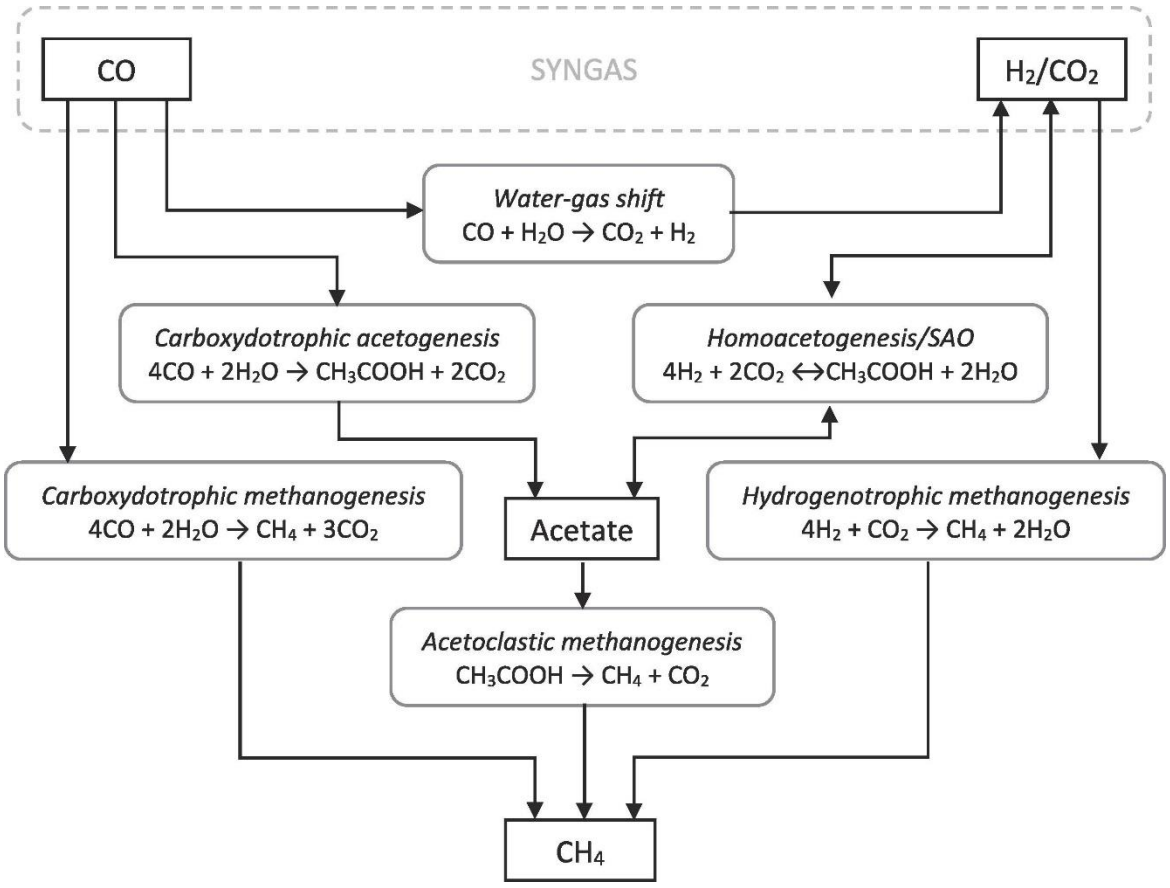


Figure 7) Pathways leading to the production of methane (adapted from Rafrafi et al.[28])

These microorganisms operate symbiotically to achieve efficient conversion, as outlined below:

- Hydrogenogens: Hydrogenogenic carboxidotrophs (H2-producing bacteria) are a group of microorganisms capable of using CO as their only energy and carbon source to produce H_2 in the absence of an electron acceptor. These bacteria can grow by oxidizing CO and reducing the protons derived from H_2O in order to produce equimolar amounts of H_2 and CO_2, analogous to the water-gas-shift reaction (WGS) previously described [29].
- Acetogenic Bacteria: Acetogens are a diverse group of anaerobic microorganisms characterized by their production of acetate from CO_2 via the reductive acetyl-CoA pathway. In this metabolic pathway, two molecules of CO_2 are reduced to a methyl and carbonyl group, which are further combined with Coenzyme A by the enzyme CODH/ACS to form acetyl-CoA [30].
- Hydrogenotrophic and Carboxidotrophic Methanogens: Hydrogenotrophic methanogens utilize H_2 and CO_2 as substrates to produce methane, playing a central role in direct methanogenesis. Similarly, carboxydotrophic methanogens leverage CO as their carbon and energy source to synthesize methane. These groups are crucial for the direct conversion of syngas components and belong primarily to the Euryarchaeota phylum [31].

- Homoacetogens: These bacteria are capable of acetate oxidation (reverse reaction) when the hydrogen partial pressure in the gas phase is low enough for the reaction to become thermodynamically favorable [32].
- Syntrophic Acetate Oxidizers: Under thermodynamically unfavorable conditions for acetoclastic methanogenesis, syntrophic acetate oxidizers convert acetate into H_2 and CO_2. This process typically occurs when acetate concentrations are high and hydrogen partial pressure is high, making acetate oxidation thermodynamically feasible [33].

This process offers advantages over chemical catalysis, such as lower energy requirements and operation under ambient conditions. In syngas biomethanation, microbial communities can either consist of pure cultures or mixed microbial consortia. Pure cultures, typically involving specific strains of hydrogenotrophic methanogens or carboxidotrophic hydrogenogens, offer higher metabolic specificity and predictable performance under controlled conditions [34]. However, they are sensitive to environmental fluctuations and require stringent operational parameters. On the other hand, mixed microbial consortia, which include methanogens and other supporting bacteria, are often employed for their robustness and ability to adapt to fluctuating syngas compositions. Despite the higher complexity of microbial consortia compared to pure cultures, the adoption of this mixed-culture approach presents a series of inherent merits such as non-sterile operation, higher adaptation capacity, higher tolerance to the impurities of the raw syngas, and resiliency after a disturbance in the operating conditions, which represent a crucial advantage when it comes to maintaining the productivity of a continuous process. Additionally, using undefined mixed microbial consortia renders continuous processes more robust, as their adaptation to the substrate selects the most efficient and effective biocatalysts leading to a long-term improved performance of the microbial consortium [35]. However, maintaining stable microbial activity requires careful control of factors like pH, temperature, and gas-liquid mass transfer.

2.2.2 State of the Art in Syngas Upgrading

The state of the art in syngas biomethanation upgrading reflects significant advancements in both the biological and engineering aspects of the process. As a sustainable alternative to conventional gas-to-energy or gas-to-chemical pathways, syngas biomethanation has been studied extensively to enhance its feasibility, efficiency, and scalability for industrial applications. Research efforts have focused on optimizing reactor configurations, improving microbial performance, and developing innovative strategies for gas-liquid mass transfer enhancement [1].

Recent innovations in bioreactor design have played a pivotal role in advancing syngas biomethanation. TBRs (Trickle-bed reactors), for instance, have been employed to provide high gas-

liquid contact areas, which is critical for the solubilization of poorly soluble syngas components like CO and H_2. CSTRs (Continuous stirred-tank reactors) and membrane reactors have also been investigated, offering unique advantages such as better mixing and selective gas separation, respectively [36-38]. These designs aim to overcome the mass transfer limitations that often hinder microbial activity and methane production [39].

On the microbial front, tailored consortia of methanogens and supporting microorganisms have been explored to enhance methane yields and process stability. Researchers have reported the successful application of mixed microbial consortia, which offer resilience to operational fluctuations and the ability to utilize multiple substrates simultaneously [40].

Operational parameter optimization remains a critical area of research. Parameters such as temperature, pressure, pH, and syngas composition significantly influence microbial activity and methane yield. Thermophilic conditions, for example, have been shown to enhance reaction rates, while the careful adjustment of H_2-to-CO_2 ratios can drive the process toward optimal methane production [26, 41]. Additionally, syngas pretreatment methods, including desulfurization and removal of contaminants like tar, have been developed to improve substrate quality and reduce microbial inhibition [4].

Despite these advancements, challenges remain in scaling up syngas biomethanation processes for industrial applications. Issues such as bioreactor design complexity, high capital expenditures, and maintaining microbial stability over extended periods continue to limit widespread adoption [42]. However, ongoing research and development efforts are expected to address these barriers, paving the way for syngas biomethanation to become a key component of sustainable carbon utilization strategies.

While existing studies have demonstrated the potential of biological syngas upgrading, several limitations persist. Conflicting findings have also been reported regarding the optimal operating pressures and temperatures, with some studies suggesting enhanced methane yields under elevated pressures, while others note microbial inhibition beyond certain thresholds. Additionally, few studies have systematically evaluated the long-term stability and resilience of microbial communities under thermophilic conditions using realistic syngas compositions.

This thesis specifically addresses these gaps by investigating the impact of key operational parameters—such as reactor pressure, temperature regime, and biocarrier selection—on the biomethanation of synthetic syngas mimicking steel industry emissions. Through systematic

experimental design and microbial community analysis, this work aims to optimize reactor performance for industrial carbon valorization applications.

2.3 Trickling Bed Reactors (TBR) in Gas Upgrading

Besides the selection of the type of the biocatalysts, a crucial parameter that should be thoroughly considered in syngas fermentation processes is the bioreactor configuration employed since it is associated with the circumvention of the poor gas-to-liquid mass transfer. With the appropriate set configuration and operating parameters of the bioreactor, an improved gas-liquid mass transfer can be achieved [43, 44].

In order to increase the mass transfer rate in this type of reactor, the goal is to decrease the size and the retention of the gas bubbles and, as a result, provide higher gas–liquid interfacial area. Compared to stirred tank reactors, trickle bed reactors (Figure 8) have been reported to provide improved gas-to-liquid mass transfer rates due to their high surface per volume ratio available from the packing material, where biofilm grows [45, 46]. This advantage comes at a lower cost because of the absence of mechanical mixing. Another major advantage of trickle bed reactors is cell retention because of the inherent ability of various microbes to form biofilms [47]. Whereas in many cases biofilm formation is connected with fouling phenomena [48], this is not the case in trickle bed reactors for syngas fermentation where the organic loading rate is low and does not allow for high microbial growth.

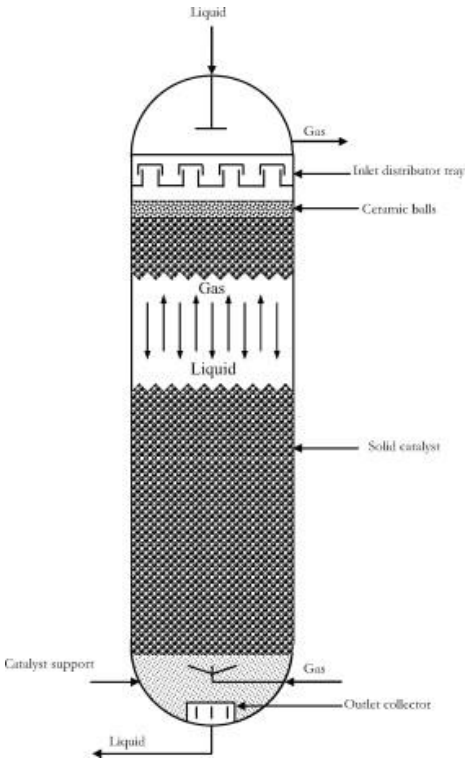


Figure 8) schematic of a TBR with countercurrent gas–liquid flow (Adapted from Mederos et al. [44])

Kimmel et al. [46] and Klasson et al. [49] were the first ones to report the potential of syngas biomethanation with trickle bed reactors and the improved methane productivity achieved compared to bubble columns. TBRs offer several advantages, including efficient mass transfer, adaptability to varying feedstock compositions, and sustainability through the use of biological processes for gas conversion. They can handle varying gas flow rates and concentrations, making them suitable for different syngas compositions. However, factors such as reactor design, packing material, gas and liquid flow rates, and temperature influence TBR performance. The basic drawback is channeling , that is the flow of the liquid through limited paths across the packed bed resulting in a non-homogenous biofilm growth in the reactor and lower CH_4 productivity rates [46].

2.3.1 Reactor Design and Operation

In the section on Reactor Design and Operation, the key factors that influence the performance of TBR in syngas biomethanation and gas upgrading processes have been discussed. The design of a TBR is critical to its efficiency, as it must promote optimal gas-liquid-solid interactions [49]. Reactor configurations, including the choice of packing material and the arrangement of the packing, affects the surface area available for microbial growth and the mass transfer between phases. Common packing materials such as structured packing, random packing, and ceramic rings are chosen based on their ability to provide a large surface area while minimizing resistance to fluid flow. The liquid distribution system also plays a key role in ensuring uniform trickling over the packing, preventing dry zones and promoting efficient microbial activity [50].

Gas flow rate and composition are crucial, as they determine the availability of syngas components for microbial conversion and influence the overall reaction rates. Temperature and pH control are also important for maintaining optimal conditions for the microorganisms involved in syngas biomethanation [51]. Additionally, the residence time of the gas in the reactor and the microbial growth rate must be carefully balanced to ensure that syngas is efficiently converted into methane.

Challenges in reactor operation include the risk of clogging and biofilm formation, which can hinder gas flow and reduce reactor efficiency. Therefore, continuous monitoring and adjustment of operating conditions, such as flow rates and microbial population management, are essential for maintaining stable reactor performance and maximizing syngas upgrading efficiency. Future innovations in TBR design will likely focus on improving reactor scalability, enhancing microbial community stability, and further optimizing operating conditions to increase the overall process efficiency.

2.3.2 Role of Internal Packing Materials

The packing materials play a critical role in improving gas-liquid contact, promoting microbial colonization, and facilitating efficient mass transfer between the gaseous syngas and the liquid phase,

where microbial processes occur. Materials such as structured metal or plastic packings provide a large surface area for microbial attachment, increasing the available sites for methanogenic microorganisms to grow and enhance the biomethanation process [52].

The internal packing can also support a stable biofilm formation, which is essential for the microbial conversion of syngas components to methane. The characteristics of the packing material, such as its surface roughness, porosity, and hydrophobicity, significantly affect biofilm formation and microbial efficiency. Flow is characterized as film or filament flow based on operating conditions, packing configuration, and fluid flow properties; liquid may traverse the bed in the form of rivulets or may get stuck in the form of pendular structures, pockets or bridges as shown in figure 9. Studies have shown that the type of packing material influences not only the biofilm stability but also the overall mass transfer rates, gas-liquid contact, and reactor performance [53, 54]. For instance, structured packings with high surface area have been shown to improve the reactor's hydrodynamics and promote better nutrient and gas diffusion to the microorganisms, thereby enhancing methane production efficiency [55].

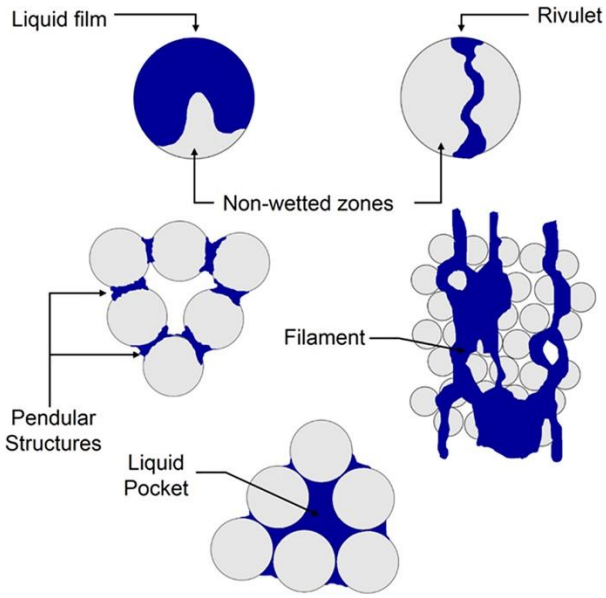


Figure 9) Different flow structures occurring in a trickle bed reactor (Adapted from Lutran et al. [56])

Moreover, the choice of packing material can also impact the flow characteristics, such as the liquid film thickness and the pressure drop across the reactor, which directly influence reactor operation and energy consumption[57]. Researchers have explored various packing materials, including ceramic, metal, and plastic-based options, each offering distinct advantages in terms of durability, ease of cleaning, and microbial support. Therefore, understanding the role and selection of appropriate packing materials is crucial for optimizing TBR performance in syngas upgrading processes [58].

2.4 Influence of Operative Parameters in Biomethanation

The efficiency of syngas biomethanation is closely tied to the careful management of operative parameters, which collectively shape the performance and stability of the process. These parameters directly influence microbial activity, mass transfer dynamics, and reactor design considerations, making their optimization essential for achieving high methane yields. Various studies have highlighted those parameters such as temperature, pressure, pH, and syngas composition need to be tailored to the specific characteristics of the microbial consortia and reactor setup [42, 59]. The interplay of these factors can significantly affect conversion efficiency and system scalability.

Reactor design and operation further amplify the impact of operative parameters. In particular, gas-liquid mass transfer is often a limiting factor in syngas biomethanation, requiring strategies like optimized flow patterns, packing materials, and reactor configurations [60].

Operational challenges, such as inhibitory compounds and fluctuations in syngas quality, also underline the importance of continuous monitoring and adaptive control. By focusing on optimizing the key parameters collectively rather than in isolation, this study seeks to establish a robust framework for scaling syngas biomethanation processes. This holistic approach ensures that the process is not only efficient but also adaptable to different industrial contexts, paving the way for broader applications in renewable energy and carbon management.

Particularly, in this study, the effects of temperature and pressure were specifically investigated as critical operative parameters influencing the biomethanation process.

2.4.1 Effect of Temperature

The temperature is one of the most influential factors in syngas biomethanation processes as it affects several aspects of the performance of mixed microbial consortia. The laws of physical chemistry tell us that the solubility of gases decreases with increasing temperature. However, with increasing temperature, microorganism activity increases and H2 and CO biomethanation efficacies are improved [61]. As mentioned already, the temperature of the broth has been reported to have an effect on the microbial interactions among members of microbial consortia as it appears to determine the predominant metabolic pathways used by the consortia.

The methanogenic archaea, the key players in methane production, are highly temperature-sensitive, with distinct optimal ranges depending on their classification as mesophilic or thermophilic species. Mesophilic conditions (typically 30–40°C) are widely used due to their stability and the abundance of mesophilic methanogens in natural and engineered systems. However, thermophilic conditions (50–

65°C) offer potential advantages, including higher reaction rates, enhanced substrate utilization, and better tolerance to some contaminants like ammonia [61, 62].

Several studies on biomethanation of CO indicate that acetate is the main precursor of the methanogenesis at mesophilic conditions, whereas H_2 is a more relevant precursor at thermophilic conditions [63, 64]. However, another possible explanation could be the fact that H_2-producing reactions become more exergonic with increasing temperatures, favoring a higher hydrogenogenic conversion of CO in thermophilic conditions [65]. Nonetheless, despite the higher conversion rate and productivity, the increase in temperature also poses certain drawbacks related to the lower solubility of the gases, which could lead to mass transfer limitations in thermophilic processes.

Maintaining a stable and uniform temperature within the reactor is critical for optimal operation. Advanced reactor designs, including efficient insulation and precise temperature control systems, were employed in this study to minimize variations and provide reliable data on the effect of temperature. The insights gained contribute to understanding how temperature optimization can enhance the scalability and efficiency of syngas biomethanation processes in industrial applications.

2.4.2 Effect of Pressure

Pressure, along with other operational conditions, are important in biological methanation because they shape the microbial community composition, which in turn affects the overall efficiency. Increased pressure can enhance the solubility of gases such as hydrogen and carbon dioxide, which are essential substrates for methanogenic microbes. Elevated pressure also affects the thermodynamics of methanogenesis, potentially increasing the rate of methane production by facilitating the activity of hydrogenotrophic and carboxydotrophic methanogens [66].

In specific applications, such as those utilizing TBR, pressure is an important operational parameter, as it can affect the mass transfer and biofilm formation on the packing material. In principle, the mass transfer from the gaseous to the liquid phase can also be enhanced by elevating the operating pressure of the reactor [67]. The pressure-induced improvements in syngas solubility and microbial growth, however, depend on the reactor design and operational conditions. Moreover, different microbial communities may respond differently to changes in pressure, suggesting that optimizing pressure must be tailored to the specific reactor system and microbial consortium used. Thus, understanding the interaction between pressure and the microbial processes is essential for optimizing biomethanation under industrial-scale conditions[68].

There are some studies on pressure's effect on biomethanation also indicate that maintaining an optimal pressure range is crucial to achieving high methane yields. It is necessary to balance the

pressure increase with the reactor's structural limits and microbial tolerance to avoid negative effects on both the system and the microbial activity [59, 69].

The partial pressure of the syngas components is a key factor in the metabolism of the microorganisms forming part of the consortium. The partial pressure of CO (P_{CO}) and/or the P_{CO} to P_{CO2} ratio can greatly affect the microbial growth and the metabolite production since some enzymes involved in the metabolic processes can be entirely or partly inhibited by substrate exposure[70]. Moreover, it has been reported that a change in P_{CO} in the gas phase can result in a shift of metabolite formation [71]. However, the application of very high pressure not only increases the operational costs but also maximizes the probability of leakage and reactor collapse [69]. Thereby, although higher methane contents can be achieved, high pressure levels can present a technical challenge for biological methanation.

2.4.3 Mass Transfer and Kinetics

Mass transfer refers to the ability of gases, such as H_2, CO, and CO_2, to diffuse through the reactor and into contact with the microbial consortium responsible for methanogenesis. In syngas biomethanation, the rate of mass transfer is often a limiting factor, as these gases are typically low in concentration in the gas phase and need to be efficiently absorbed into the liquid phase to be utilized by the microorganisms. TBRs are gas phase reactors filled with high specific surface area packing material over which liquid medium is trickled. The packing material and trickling simultaneously promote gas–liquid mass transfer and the formation of biofilm, which allows the retention of slow growing anaerobes in the reactor [72]. The presence of an efficient mass transfer system, such as optimized packing materials in TBRs or appropriate reactor design, can significantly enhance the contact between the gases and microbes, thus improving the overall efficiency of the process.

Although biofilm gives trickle bed reactors an advantage in gas fermentation, the compounds in the gas phase do need to be transported within the biofilm to reach the microbes that will eventually consume them. This transport occurs by diffusion, and at rates much slower than those reported for gas–liquid mass transfer [73]. However, the major mass transfer resistance observed during syngas fermentation processes is the mass transfer across the gas-liquid interface [5]. It is thus plausible to hypothesize that gas–liquid mass transfer is the sole bottleneck in the syngas biomethanation process.

To have a better understanding of the mass transfer rate in the media, it is important to know the volumetric mass transfer coefficient, K_{La} (s^{-1}), which can be determined using the following equation:

$$\text{Overall mass transfer rate} = k_{Lai} * (H_{i,\,cp} * P_i - C_{i,\,L})$$

It is linked to the mass transfer coefficient k_{Lai} [1/s], which can be influenced by reactor type and operating parameters. It is also correlated to the concentration gradient ($H_{i,\,cp}*P_i - C_{i,\,L}$), with Hi, cp [mol/(L.bar)] being the Henry's law constant, P_i [bar] the partial pressure of the gas and $C_{i,\,L}$ [mol/L] its concentration in the liquid phase. It appears that increasing the partial pressure of the substrates by increasing the overall pressure of the process can lead to better transfer performances. Therefore, a higher pressurized process can lead to intensified methane production, which is a significant step towards an industrial application. However, with an intensified pressurized process, another limiting step of syngas biomethanation could be CO inhibition. An increase in CO partial pressure and thus in CO transfer rate can lead to high soluble CO concentrations. These high soluble CO concentrations could be close to the inhibition level for the different microbial groups involved into biomethanation reactions [74, 75].

As previously commented, it has also been observed that the adaptation of the microbial culture to high CO pressures can be achieved by gradually increasing the pressure in the system [76]. It is therefore important to evaluate the kinetics of the reaction and have a good correlation between the substrate diffusion into the medium and the specific substrate consumption rate.

Kinetics governs the rate at which biochemical reactions occur within the microbial community. In the case of syngas biomethanation, the kinetics of hydrogenotrophic and carboxydotrophic methanogenesis are essential to determine how quickly the microbes can convert the substrates into methane. The rate of reaction is affected by several factors, including the concentration of substrates, the activity of the enzymes involved, and the growth rate of the microbial species. Understanding and modeling the reaction kinetics is key to predicting the performance of the bioreactor under different operational conditions. For instance, if the mass transfer is optimized but the reaction kinetics are slow, the overall production rate of methane may still be suboptimal.

Both mass transfer and kinetics are interrelated and must be considered together when designing or optimizing a bioreactor system. If the mass transfer is insufficient, even the most efficient microbial consortia may not be able to effectively use the available syngas components. Conversely, if the kinetics are not favorable, the reaction will proceed at a slower rate, leading to lower methane yields. Therefore, optimizing both aspects is essential for improving the efficiency of syngas biomethanation. In practice, this may involve adjusting operational parameters such as reactor flow rates, pressure, temperature, and gas-liquid contact area [77, 78].

2.5 Metagenomic Insights into Biomethanation

Metagenomics has emerged as a powerful tool for understanding the complex microbial communities involved in biomethanation processes. By analyzing the collective genetic material of microbial

consortia, metagenomics provides a comprehensive view of the microbial diversity, functional potential, and interactions that drive methane production from syngas. This approach enables researchers to identify key microbial players, pathways, and genes involved in the methanation process, shedding light on the mechanisms underlying microbial adaptation and resilience in different environmental conditions [79, 80].

In biomethanation, the microbial community is typically composed of bacteria and archaea that work synergistically to convert syngas components into methane. Metagenomic studies have revealed that hydrogenotrophic methanogens, such as those belonging to the *Methanobacterium* spp. and Methanosarcina genera, play a central role in direct methanogenesis by utilizing H_2 and CO_2. Additionally, carboxydotrophic methanogens, capable of utilizing CO as a substrate, have been identified in syngas biomethanation systems [52]. Metagenomic analyses have also highlighted the importance of acetogens, which produce acetate as an intermediate, and syntrophic bacteria that facilitate interspecies electron transfer under specific conditions [81]. The integration of metagenomic data with microbial ecology and biochemical analysis has provided insights into how these groups interact and optimize energy utilization in the system.

One significant advantage of metagenomics is the ability to monitor microbial dynamics in response to varying operational conditions, such as temperature, pressure, and gas composition. For instance, shifts in microbial community composition have been observed when transitioning from mesophilic to thermophilic conditions, with thermophilic methanogens demonstrating enhanced activity at higher temperatures [61]. Similarly, metagenomic studies have shown that high pressures influence microbial diversity, favoring species that are better adapted to elevated gas concentrations and pressures. These insights are invaluable for optimizing reactor design and operation, as they allow for tailoring conditions to enhance the activity of specific microbial groups [59].

Despite its advantages, metagenomics also has limitations. While it provides a wealth of information on the genetic potential of microbial communities, it does not directly reveal the activity or expression levels of the identified genes.

3. Materials and Methods

This chapter outlines the experimental framework used to investigate the biomethanation of syngas in trickling bed reactors (TBR). The focus of the study is to examine the influence of operative parameters such as temperature and pressure on methane production, using a mixed microbial consortium. Detailed descriptions of the materials employed, the design and operation of the experimental setup, and the analytical methods used for monitoring and evaluating the processes are provided.

The experimental setup was carefully designed to replicate industrial conditions, ensuring the relevance and scalability of the results. Two TBRs were operated under different conditions to explore the effects of varying parameters on the efficiency of syngas conversion.

Analytical methods were employed to monitor the performance of the reactors and to quantify the microbial activity, gas composition, and product yields. Additionally, metagenomic analyses were conducted to assess the microbial community structure and dynamics, providing insights into the microbial mechanisms underlying the observed outcomes.

This chapter provides a comprehensive overview of the methodologies employed, enabling the reproducibility of the study and facilitating a deeper understanding of the factors influencing syngas biomethanation in TBR systems.

3.1 Experimental Setup

The experimental setup for this study was designed to investigate the biomethanation of syngas in trickling bed reactors (TBR). The bioreactor scheme is shown in figure 10. The bioreactor design refers to the three-phase system with a countercurrent flow configuration. Each bioreactor was packed with certain types of biocarrier. The total biocarriers in each reactor was divided into three segregated zones.

The reactors were equipped with a temperature control system consisting of a jacketed design. A liquid, maintained at a specific temperature, was circulated through the jacket to ensure precise temperature regulation within the reactor.

Each reactor was equipped with a temperature probe positioned at the bottom to continuously monitor the internal temperature, ensuring accurate readings and effective temperature control throughout the experiments. Additionally, a pressure gauge was installed at the top of the reactor to measure and monitor the internal pressure, providing critical data for maintaining safe and optimal operating conditions.

The gas feed system involved individual gas cylinders to create a synthetic syngas with a controllable composition tailored for experimental needs based on the table 3. The flow rates of each gas component were precisely regulated using mass flow meters, ensuring accurate and consistent delivery. The mixed gases were then injected into the reactor at the bottom, facilitating thorough mixing and efficient contact with the microbial consortia. Gas flow rates were measured using a MilliGascounter (Ritter Apparatus, Germany).

Presented in figure 11 is an image depicting the actual experimental set-up utilized in our biomethanation studies, showcasing the arrangement of reactors, control systems, and monitoring instruments.

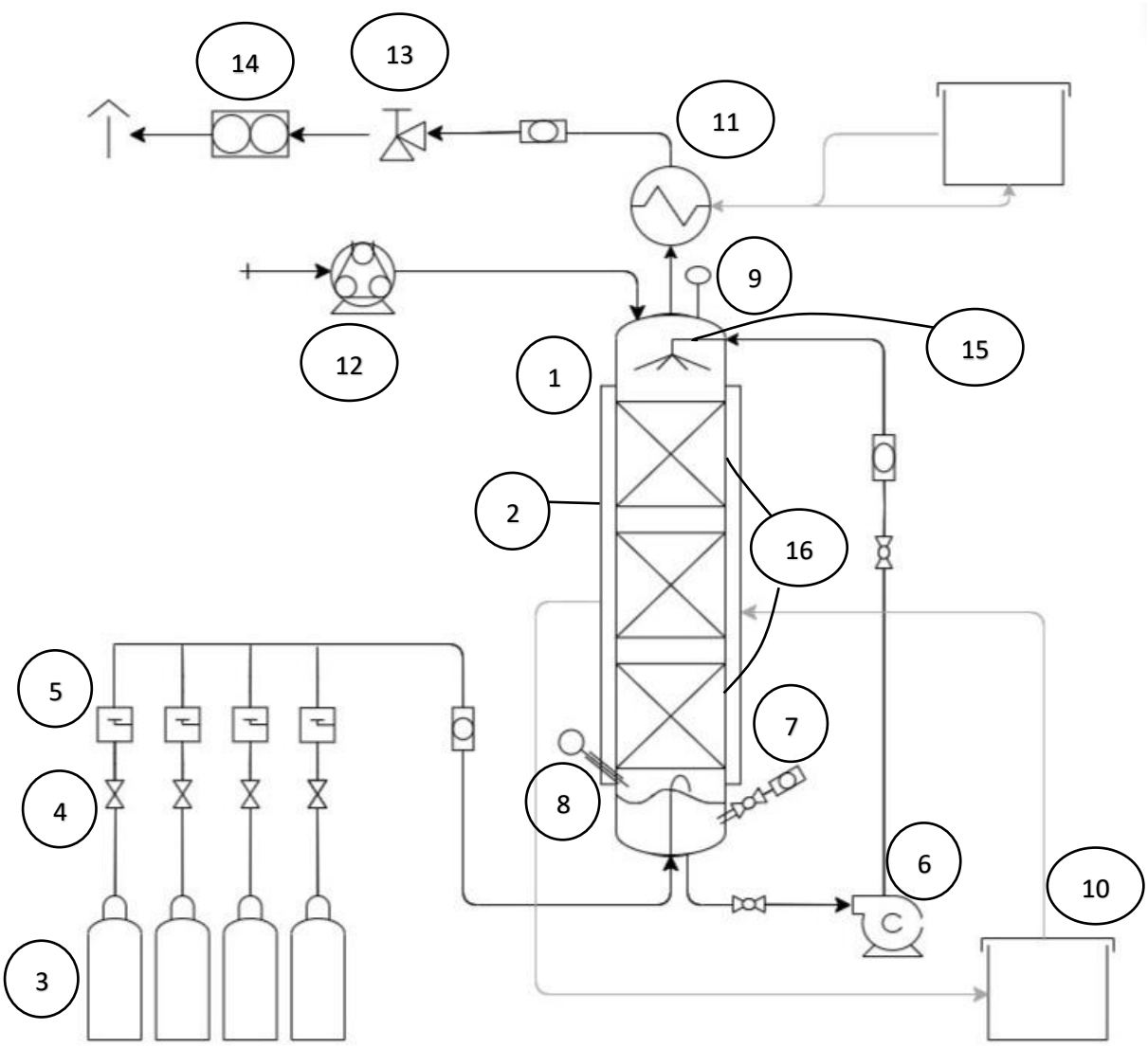


Figure 10) experimental set-up, 1) reactor vessel, 2) Heating jackets, 3) gas Cylinder, 4) Regulating valves, 5) Mass flow meters, 6) Pump, 7) Liquid port, 8) Temperature probe, 9) Pressure gauge, 10) Heat exchanger reservoir, 11) Condenser, 12) liquid in pump, 13) pressure relief valve, 14) gas meter, 15) nozzle, 16) metal rack

Table 3) Gas inlet compositions

Inlet Gas	H2/CO2 (%)	COG (%)	BFG (%)	BOF (%)
H_2	80%	52%	8%	0%
N_2	0%	41%	44%	20%
CO	0%	5%	25%	60%
CO_2	20%	2%	23%	20%

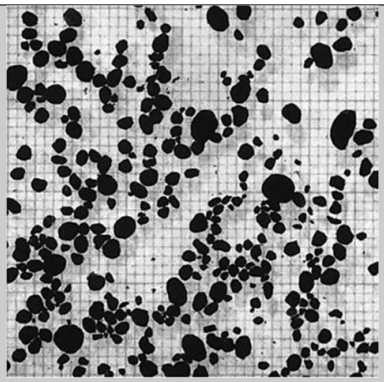
Figure 11) experimental set-up

3.1.1 Inoculum and liquid media

The tests carried out for this study were performed under mesophilic conditions (36˚C), using anaerobic granular sludge from a full scale UASB plant treating fruit processing wastewater (Lassonde Inc., Rougemont, QC, Canada). The other sludge used in this study in thermophilic condition was collected from ADM (Archer Daniel Midland, Candiac, QC, Canada).

Feeding characterization and operational conditions of the mentioned inoculum UASB reactor is presented in Table 4 The granular structure was blended via a blender with the speed or 2000 rpm for 4 min. The biomass was re-suspended in 0.05 M phosphate buffer at pH 7.5. COD measurements presented in Table 4 correspond to the total COD content of the initial sludges used for inoculation. COD serves as an indicator of the amount of organic matter available for microbial metabolism, reflecting the potential of the inoculum to support biomethanation activity. In this study, COD values were expressed in mg O_2/L. These values allowed us to assess the organic richness of the Lassonde and ADM anaerobic sludges prior to reactor inoculation and to standardize initial biomass loading across different experimental phases.

Table 4) Feeding characterization and operational conditions of the sludges

Effluent treated	Fruit juice factory (Lassonde)	ADM
Digester volume capacity (m^3)	400	12453.69
Retention time (average)	24 h	6-8 d
pH in digester	6.8-7.2	6.8–7.1
Total COD (wastewater in) (mg/L)	4500-7000	21000
Soluble COD (wastewater in) (mg/L)	4500-6000	10500
Total COD (effluent) (mg/L)	300-500	8230
Soluble COD (effluent) (mg/L)	200	307
Ammonia (effluent) (mg/L)	≤1	unknown
Granules (square = 1 mm^2)		

In liquid media, the composition and preparation of the culture medium used for microbial consortia in the biomethanation process are carefully defined to ensure the availability of essential macro- and micronutrients, supporting microbial growth and activity throughout the experiments. The selected media were designed to provide essential nutrients, and trace elements necessary for the growth and metabolic activity of the microbial populations involved in the syngas conversion process. The liquid media also included the addition of phosphate buffer at pH 7.5. This buffer was essential to maintain a stable pH environment within the reactor, as fluctuations in pH can adversely affect the metabolic processes of the microbial consortia, particularly methanogenesis. The phosphate buffer helps to prevent acidification or alkalization of the media. The total liquid volume added to the reactor was 5L.

The primary media used in these experiments were tailored to support the specific metabolic pathways of both hydrogenotrophic and carboxydotrophic methanogens, as well as homoacetogens. The base medium consisted of a balanced mixture of macronutrients, including nitrogen, phosphorus, and potassium, supplemented with essential trace elements like iron, zinc, and magnesium. The retention time of the liquid media in the reactor is maintained at 28 days. To ensure the consistency of the nutrient supply, a specific volume of the liquid media is manually removed from the reactor twice a week and replaced with fresh media. This process helps sustain the microbial activity and stability of the system over the course of the experiments. The reactors initially were filled with 5L of liquid media.

For clarity and reproducibility, the composition of each Liquid medium is detailed in Table 5. This table lists the concentration of each component, along with any special preparation or sterilization steps necessary before use.

Table 5) Composition of liquid media

Phosphate buffer pH 7.5, 0.05M		
buffer TA	Molecular weight	Concentration [mg/L]
K_2HPO_4 (mg/L)	174	4000
Na_2HPO_4 (mg/L)	142	2700
$NaH_2PO_4.H_2O$ (mg/L)	138	1100
Nutrient solution		
KH_2PO_4	136	500
$MgCl_2$ .6 H_2O	203	330
NaCl	58	400
NH_4Cl	53	1000
$CaCl_2$ .2 H_2O	147	50
Trace element solution (from DSMZ)		
$FeCl_2$ .4 H_2O	199	1.5
$ZnCl_2$	136	0.07
$MnCl_2$ .4 H_2O	198	0.1
H_3BO_3	62	0.006
$CoCl_2$ .6 H_2O	238	0.19
$CuCl_2$ .2 H_2O	170	0.002
$NiCl_2$ .6 H_2O	238	0.024
Na_2MoO_4 .2 H_2O	242	0.036

3.1.2 Description of the TBR Reactor

In this section, a detailed description of the Trickling Bed Reactor (TBR) utilized in our experiments is provided. The reactor is designed to facilitate efficient gas-liquid contact, essential for the biomethanation process. The reactors consist of two extended vessels attached vertically, creating a single, continuous system. The TBR is constructed from stainless steel, ensuring durability and resistance to corrosion, with a total working volume of 38L. The reactor features a window in each vessel, providing a clear view of the internal processes. These windows allow for direct observation of biofilm growth on the biocarriers and ensure the proper functioning of the trickling system. This visual access is crucial for monitoring the health and distribution of the microbial population, as well as for identifying any potential issues with biofilm formation or fluid distribution within the reactor (Figure 12).

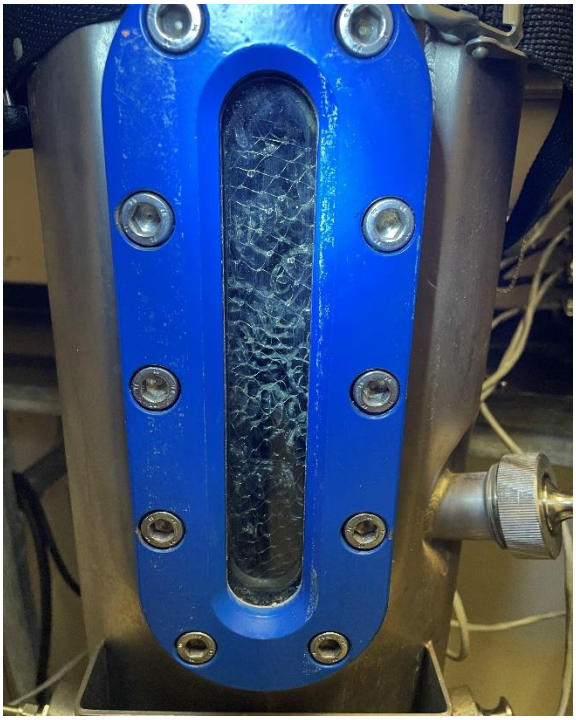


Figure 12) reactor's window

The reactor features an internal rack system, custom-designed to support the packing material and enhance mass transfer between phases. This internal rack is strategically divided into three zones. One of the main reasons for incorporating the internal rack within the reactor is to facilitate the study of microbial populations in each zone and observe the differences over time. This setup allows for the sampling of specific sections, enabling a detailed analysis of how microbial communities evolve and adapt in different reactor zones during the biomethanation process. A nozzle at the top facilitates the even distribution of the gas phase, optimizing contact with the liquid media. Figure 13 shows the 3D render of the reactors and internal rack.

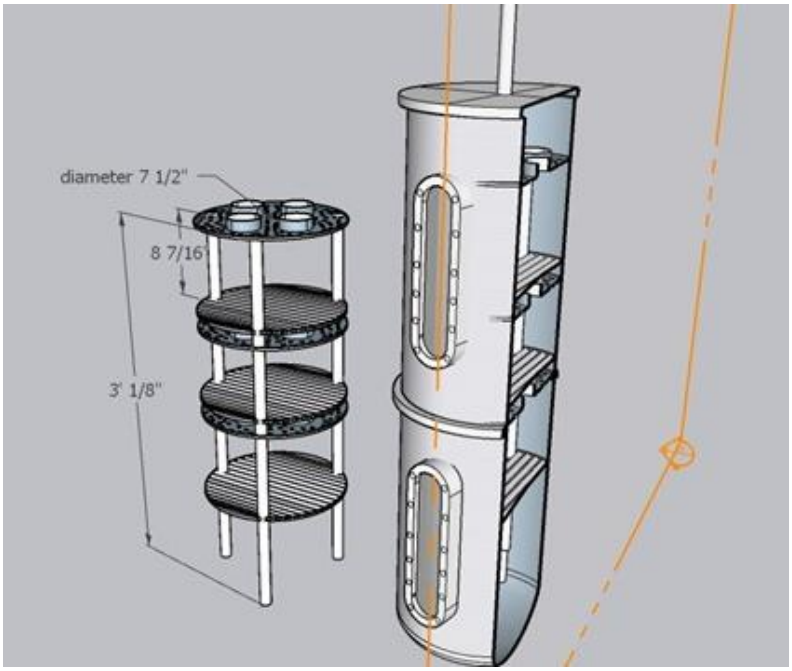


Figure 13) 3D render of the reactors and internal rack

Table 6) configuration of the reactors

Reactor dimensions	
Max working volume of each vessel [L]	26
Inner diameter [mm]	200
Inner height [mm]	1220
D_i/H_i	1:6.0
Total volume [L]	38
Internal rack dimensions	
Hight	41 in
diameter	7.5 in
Hight of first bed support the bottom	~9 in
Hight of each zone	~10 in

Additionally, the reactor is equipped with a temperature control system, featuring a jacket through which temperature-controlled liquid circulates, maintaining a consistent operating temperature. A temperature probe is positioned at the bottom, and a pressure gauge is mounted at the top, providing precise monitoring and control of the reactor's internal conditions.

This innovative design allows for effective management of the process parameters, contributing to the reactor's overall performance in converting syngas into methane. The reactor's design

considerations and operational features are critical in achieving the study's objectives, ensuring reliable and reproducible results.

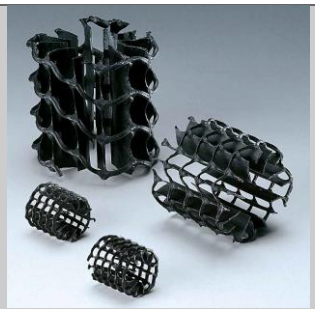
3.1.3 Packing Materials Used

Here we detail the two types of packing materials employed in the reactor, which primarily differ in their surface area. These materials are lightweight and cost-effective plastic packings, chosen for their practical advantages in facilitating efficient microbial growth and gas-liquid contact. The specific characteristics of each packing material, including surface area and other relevant properties, are summarized in the accompanying table 7 for a comprehensive comparison.

Both packing materials utilized in the reactor were procured from RVT Process Equipment in Germany. The first type, RFK 25L, is a Biological Random Packing made from PE-recycled material, designed to enhance microbial adhesion and gas exchange. The second type, HF25-7-PP, is a 1" Hiflow Ring Tower Packing crafted from polypropylene (PP), known for its robust structural integrity and efficient mass transfer properties. These materials were selected for their suitability in promoting optimal biomethanation processes within the reactor.

Each reactor was packed with an identical volume of internal packing material, distributed evenly across three separate zones. In each zone, the same volume of packing was encased in a plastic net to ensure uniformity and facilitate ease of handling and analysis, as illustrated in figure 14.

Table 7) Internal Packing Characteristics

Packing Type	Packing Shape	Material	Weight (g)	bulk density kg/m³	surface area m²/m³	void fraction in %
HF25-7-PP 1" Hiflow Ring Tower Packing		Poly Propylene	1.96	90	214	91
RFK 25L RFK Biological Random Packing		Recycled Poly Ethylene	1.7	71	312	90

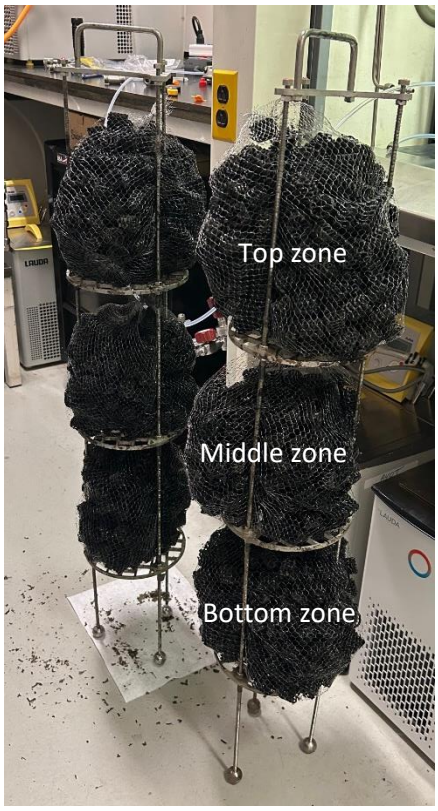


Figure 14) internal packing distributed in 3 zone on the internal rack.

3.2 Experimental Design and Protocols

In the Experimental Design and Protocols section, the experimental framework for investigating syngas biomethanation is outlined, detailing the systematic approach taken to evaluate the influence of various operational parameters on methane production. This section will describe the design of experiments, including the selection of conditions, controls, and the step-by-step protocols. The methods for sampling, monitoring, and data collection will also be explained to provide a comprehensive understanding of the experimental setup and procedures.

This section describes how the experiments were conducted across three distinct phases, each aimed at optimizing the syngas biomethanation process. These phases were designed to systematically assess and refine various parameters, including the impact of internal packing, reactor pressure, and temperature, to enhance methane production efficiency. Each phase builds upon the findings of the previous one, incorporating iterative improvements to approach optimal performance.

3.2.1 Phase 1: Internal Packing

In the first experimental phase, the focus was on evaluating the impact of different internal packing materials on syngas biomethanation efficiency. Two types of packing materials were selected: RFK Biological Random Packing (EFK) for Reactor 1 (R1) and Hiflow Ring Tower Packing (HF) for Reactor 2 (R2). Both reactors were packed with an equal volume of packing material, ensuring a total working volume of biocarriers at 18 liters.

For inoculation, the same microbial consortia (Lassonde) were used in both reactors to maintain consistency. The initial volatile suspended solids (VSS) concentration was standardized at almost 5 g/Lpbd. This setup ensured that any observed differences in methane production could be attributed primarily to the type of packing material rather than variations in microbial activity.

The biocarriers were thoroughly mixed with the microbial sludge before inoculation into the reactors. The reactors were then operated at a controlled temperature of 36°C and a pressure of 1.5 atm (in the bottom) to facilitate microbial enrichment. This phase was critical for determining the influence of packing material characteristics, such as surface area and material properties, on the overall performance of the biomethanation process.

The reactors were kept under these operational conditions for weeks so as to develop a stable biofilm within the trickle bed column. Afterwards, the syngas inflow rate was gradually increased and a steady

state was established in each of the reactors at each individual syngas inflow. This step allowed for the evaluation of the reactors' performance under increasing flow rates, helping identify the point at which the reactors could operate at their optimal capacity. The reactors were operated for a continuous period of four months, testing different feed compositions, during which data were routinely collected to monitor the performance and stability of the biomethanation process. Regular sampling and analysis were conducted to track key parameters such as methane production, microbial activity, and the condition of the packing materials.

3.2.2 Phase 2: Pressure Variation

In the second phase of the experiment, which focused on pressure variation, after four months of reactor operation, the reactors were opened for analysis. Figure 15, showed the how the biofilm grows on the biocarriers after four months of inoculation.

Figure 15) top zone of biocarriers after 4 months of experiment for each reactor (RFK – black vs. HF – white)

The data collected during this period were thoroughly reviewed, and based on the results, the best-performing packing type was selected for further investigation. Once the packing type was finalized, the reactors were operated under two different pressure conditions: 1.5 atm for R1 and 2.5 atm for R2. The upper pressure limit of 2.5 atm was selected based on the safety criteria of our reactor system, ensuring that it remained within the designed operational limits.

For the microbial consortia, the selected biocarriers with biofilm were carefully collected. To maintain consistent microbial activity, half of the initial volatile suspended solids (VSS) from the fresh sludge were added to the biocarriers, and an equal amount of new biocarriers was introduced. This mixture was then divided into two portions, each packed into a separate reactor. This approach ensured that the same VSS concentration (5.1 g/Lpbd) was maintained in both reactors, allowing for a fair

comparison between the different pressure conditions. The reactors were then run for several months with different feed compositions to evaluate the effect of pressure on the biomethanation process.

After inoculating the reactors with the selected microbial consortia and biocarriers, the reactors were kept under the specified pressure conditions for several days to allow the biofilm to stabilize within the trickle bed column. Subsequently, the syngas inflow rate was gradually increased to allow the system to adapt to the new operational conditions. This gradual increase in the inflow rate helped establish a steady-state operation in each of the reactors under two different pressures (1.5 atm and 2.5 atm). Monitoring of reactor performance continued until a stable methane production rate was observed, ensuring that the reactors were operating at the optimal conditions for each pressure.

3.2.3 Phase 3: Temperature Variation

In the third phase of the experiment, which focused on temperature variation, after selecting the optimal pressure, we reinoculated the reactors to investigate the effects of two different temperature conditions: 36°C for mesophilic (R1) and 56°C for thermophilic (R2) environments. To ensure a consistent microbial population, we mixed thermophilic sludge with the biocarriers from the previous phase, which had already undergone the best pressure treatment. To ensure a consistent microbial population, again, we added new biocarriers (18L) to the already biofilm-coated biocarriers from the previous phase, which had already undergone the best pressure treatment (18L from R1).

To reach 5 g/Lpbd of inoculum from the total 36L of biocarriers, we combined half VSS from fresh mesophilic sludge (blended Lassonde) with half of VSS from fresh thermophilic sludge (ADM). The resulting inoculum was divided into two portions, which were packed into both reactors, one set to mesophilic conditions at 36°C and the other to thermophilic conditions at 56°C. By this approach, we were able to evaluate the impact of different temperature regimes on the biomethanation process under the previously optimized pressure conditions.

3.3 Metagenomic Analysis

The microbial community structures in the TBRs were monitored by analyzing the biofilm developed on the biocarriers, as well as the populations present in the circulating media. DNA was extracted from the sludge and the reactor samples (approx. 250 mg samples) using a DNeasy PowerSoil Pro kit (Qiagen, USA) and following the manufacturer's instructions. DNA quantity assessments were carried out using the Quant-iT PicoGreen assay (Fisher Scientific Ltd).

Metagenomic sequencing libraries were prepared with 1 ng of DNA using the Nextera XT DNA Library Preparation Kit from Illumina (San Diego, CA, USA.). Equimolar amounts of the libraries were pooled and sequenced on an Illumina NovaSeq 6000 S4 PE150 system on a 2 × 150 bp configuration at the

Centre d'expertise et de services Génome Québec. The generated sequencing reads were processed, assembled and annotated using the ShotgunMG bioinformatics pipeline. The taxonomic diversity of the samples was determined based on *rpoB* gene abundance profiles as described in Tremblay et al. article [82].

3.4 Metabolic Activity Testing

Specific activity of a biomass sample measures the rate at which a specific molecule is used by a certain quantity of microorganisms. Carboxydotrophic specific activity tests were performed on the initial blended Lassonde and ADM. Sludge is sampled from the reactor with a plastic syringe and dispensed in a plastic bottle flushed with N_2 gas.

3.4.1 Protocols for Activity Measurement

The protocols for the activity measurement tests involved systematic sampling and analysis to assess the performance of the microbial consortia in microcosms. Key steps included preparing the samples under controlled conditions, measuring gas production using a gas chromatograph, and monitoring the concentration of methane and other gases over time. The tests were conducted following standard operating procedures to ensure consistency and reproducibility across all phases of the experiment [83].

For soluble substrates, tests are conducted in serum bottles partially filled with diluted biomass. Concentration of the biomass should be low enough for the biological substrate consumption to be significantly lower than the inter-phase (liquid to bacteria) transfer rate [84].

Periodically, the liquid phase is sampled using a syringe for quantification of the specific substrate by High-performance liquid chromatography (HPLC) or gas chromatography (GC). The values are used to calculate the instantaneous quantity of the specific substrate in the bottle.

The volumetric substrate activity is estimated by calculating the average depletion rate of the substrate in a serum bottle containing biomass and the diluted substrate. Specific activity is estimated by dividing the volumetric activity by the amount of biomass in the bottle. Volumetric activity can be estimated from the average of the calculated slope of the substrate concentration versus time curve for each replicate. Since we are interested in the maximal volumetric activity, we look for the maximal slope over a certain period of the test. At first, estimation of the maximal slope, using linear regression techniques involve the rejection of non-relevant data points. In choosing data points lying on the straight line of the maximal slope, points corresponding to a starting or ending plateau (lag phase or starvation) should be discarded.

3.4.2 Activity Tests

The acetoclastic, hydrogenotrophic, and carboxydotrophic (CO-oxidizing) specific activity tests were carried out with acetate H_2/CO_2 and CO substrate respectively in 60 mL serum bottles. The bottles were filled with 20 mL of the inoculum diluted with 0.05 M phosphate buffer at pH 7.5 to an initial concentration of 2 g volatile suspended solids (VSS)/L and 5 gVSS/l for acetoclastic activity. To establish anaerobic conditions the bottles were capped, sealed with butyl rubber stoppers and flushed with required gas for 2 min. Specifically, for CO activity test, bottles were flushed with N_2, afterwards CO was injected into the bottles under anaerobic conditions using a gas tight syringe to obtain the required CO concentrations in the headspace. The bottles were immediately placed in dark environmental conditions in a rotary shaker (New Brunswick, Edison, NJ) controlled thermostatically at 35 ± 3 ºC and operated at 200 rpm to help the liquid-gas mass transfer.

The hydrogenotrophic activity tests were performed similarly, using as substrate instead of acetate, headspace filled with CO_2/H_2 (80/20% vol./vol.) pressurized at 20psi and by shaking at 400 RPM to ensure the gas transfer between the headspace and liquid.

And for investigating the activity of Acetate as a substrate, prepared bottles were flushed with N_2/CO_2 (80%/20%) with no pressure and add 200ul of 300 mg/L acetate substrate solution in the bottles. The acetate stock solution composition is presented in table 8. Prepared bottles were incubated and gyrated at 100 RPM.

Table 8) Acetate stock solution (about 300 g/L)

Components	Weight (g)
Ammonium acetate	12.4 g
Potassium acetate	16.4 g
Sodium acetate	18.3 g
Glacial acetic acid	6.64 g

In Phase 1, where Lassonde served as the initial sludge source, the activity test bottles were incubated at 35°C, providing conditions suitable for mesophilic microbial activity. Conversely, in Phase 3, the initial sludge comprised a mixture of Lassonde, mesophilic, incubated at 36 and ADM which is a thermophilic sludge, necessitating incubation at 52°C to accommodate and enhance the activity of thermophilic microorganisms.

During the incubation period the bottles were sampled for CH_4, H_2 and CO at regular time intervals depending on the initial CO concentration until the substrate was totally depleted. At the end of each

assay liquid samples from each bottle were analyzed for the presence of volatile fatty acids (VFA) and alcohols. To ensure the reliability and accuracy of the experimental results, each experiment was conducted in triplicate bottles. This approach was implemented to minimize potential errors and variability, thereby enhancing the statistical validity of the findings and providing a more robust dataset for analysis [83].

3.5 Analytical Methods

To quantify the biomass concentration, two 35 mL aliquots of liquid suspension were sampled from the reactor and centrifuged at 10,000 rpm for 10 min at 4 °C. The pellets were resuspended in 2 mL of distilled water and the solid concentration was quantified using chemical oxygen demand (COD) measurements determined according to standard methods [85].

Measurements of H_2, N_2, O_2, CO, CH_4 and CO_2 were made on an Agilent 7820 gas chromatograph (Wilmington, DE) coupled to a thermal conductivity detector (TCD). Three hundred µL of headspace gas were sampled and injected on a 2 m × 2 mm I.D. ShinCarbon ST packed column from RESTEK. The column was heated at 40 °C for 5 min then raised to 200 °C at a rate of 20 °C min−1 maintained for 2 min. Argon was used as carrier gas at a flow rate of 10 mL min−1. The injector and detector were maintained at 125 °C and 250 °C respectively [86].

Volatile fatty acids (VFA, i.e., acetic, propionic and butyric acids) were measured on an Agilent 6890 gas chromatograph (Wilmington, DE) equipped with a flame ionization detector (FID) on 0.2 µL samples diluted 1:1 (vol./vol.) with an internal standard of iso-butyric acid in 6% formic acid, directly injected on a glass column of 1 m × 2 mm Carbopack C (60–80 mesh) coated with 0.3% Carbowax 20 M and 0.1% H_3PO_4. The column was held at 130 °C for 4 min. Helium was the carrier gas fed at a rate of 20 mL min^{-1}. The injector and the detector were both maintained at 200 °C [86].

To analyze dissolved Carbon Monoxide and Hydrogen in the reactor, an approximate 8 mL volume of liquid culture medium to be tested was transferred with a gas-tight syringe into a 20 mL gas-tight glass vial, which was pre-tared, both empty and fully filled with water, including stopper and cap and flushed with N_2. Once the sample transferred, the vial was weighted. The difference between this weight, empty tare and filled tare determined accurately the liquid sample volume and the headspace volume, respectively. The vials were then placed in a 100 °C thermostatic water bath during 30 min so that all dissolved CO (dCO) and H_2 (dH_2) was released in the headspace. Afterwards the CO and H_2 content in the headspace was determined by GC, allowing for the calculation of the dCO and dH_2 concentration [87].

3.5.1 Gas Composition Analysis

For gas composition analysis, the primary objective was to monitor and quantify the composition of gases produced during the biomethanation process in the reactors. This analysis is essential to evaluate the efficiency of the biomethanation reaction, as well as to understand the conversion dynamics of syngas into methane, and the presence of other gases such as CO_2, H_2, and CO. Gas samples were collected daily from the reactors using gas-tight syringe, ensuring that the composition of gases within the reactors was accurately captured over time. The samples were then analyzed using gas chromatography (GC), a precise and reliable method for detecting and quantifying individual gases in the mixture. The GC setup was configured to measure specific gas components.

The gas composition data obtained by massflow controllers and gasmeters were used to calculate key performance indicators such as methane yield and conversion efficiency under different operating conditions.

Therefore, several key parameters were calculated to assess the performance of the biomethanation process. The percentage of gas in and gas out was determined to evaluate the efficiency of the gas flow through the reactor. The percentage of CO and H_2 consumed was calculated based on the difference between the gas composition entering and exiting the reactor. These values can be expressed using the following equations:

$$Percentage\ of\ Gas\ Out = \frac{Gas\ out}{Gas\ in} \times 100$$

$$CO\ consumed = CO\ In - CO\ Out$$

$$H_2\ consumed = H_2\ In - H_2\ Out$$

This comprehensive gas analysis is critical to understanding the impact of various operational parameters, such as pressure, temperature, and packing materials, on the biomethanation process.

3.5.2 Methane Production Measurement

The methane yield was also calculated by comparing the amount of methane produced per chemical oxygen demand (COD) consumed. COD was used in this study as a measure of the amount of available substrate for microbial consumption. It serves as a proxy for the biodegradable material in the syngas that could potentially be converted to methane during the biomethanation process. By tracking the COD, we can effectively quantify the organic matter entering the reactor and assess how efficiently the microbial consortia in the reactor are converting the components of syngas (such as CO, H_2, and) into methane. Using COD as the basis for calculating methane yield provides an accurate comparison of the reactor's performance under different conditions, as it normalizes the data for the available

substrate, irrespective of other variables. This allows for the determination of the efficiency of methane production relative to the materials consumed, which is crucial for evaluating the effectiveness of the biomethanation process in terms of both substrate utilization and methane production.

This is a critical metric for evaluating the efficiency of methane production. The following equation was used:

$$Methane\ Yield = \frac{CH_4\ Produced\ (mol)}{COD\ Consumed\ (g)} \times 100$$

Lastly, methane production rate was calculated in terms of mol of methane produced per square meter of reactor surface area per day (mol/m²·d) to assess the productivity of the biomethanation process:

$$Methane\ Production\ Rate = \frac{CH_4\ Produced\ (mol)}{Reactor\ Surface\ Area\ (m^2)} \times Time\ (days)$$

4. Results and Discussion

In this section, we present a comprehensive analysis of the experimental data obtained from the various phases of the study. This section will detail the outcomes related to the influence of packing materials, pressure variations, and temperature conditions on the performance of TBRs in biomethanation. Key performance indicators such as gas composition, methane yield, and conversion efficiency will be critically examined to understand the underlying mechanisms and trends observed.

We will begin by discussing the impact of different packing materials on biofilm formation and gas-liquid mass transfer, evaluating how these factors influence the overall efficiency of methane production. Following this, we will explore the effect of varying pressure conditions on the microbial consortia's activity and the system's capacity to handle increased syngas inflow rates, thereby assessing the optimal operating pressure for enhanced methane yield. The third phase will focus on the temperature's role in biomethanation, comparing the results from mesophilic and thermophilic conditions. We will also present the results of the activity tests and metagenomic analysis conducted during the study. The activity tests will provide insights into the microbial consortia's performance under different operational conditions, highlighting their adaptability and efficiency in methane production. Additionally, the metagenomic analysis will offer a detailed examination of the microbial community structure and dynamics across the various phases. By comparing the genetic profiles from different conditions, we aim to identify key microbial players and their functional roles in the biomethanation process.

Throughout this section, we will interweave our findings with existing literature, highlighting consistencies or deviations, and providing a critical interpretation of the results. This discussion will culminate in identifying the optimal conditions for maximizing methane production, contributing to the broader understanding of biomethanation processes and their potential applications in industrial settings.

4.1 Phase 1: Effect of Internal Packing

In this phase of the experiment, we evaluated the performance of two reactors packed with different types of biocarriers under identical operational conditions. Both reactors were operated at a pressure of 1.5 atm in the bottom and a temperature of 36°C. The liquid residence time was maintained at one month, with the liquid recirculation pump running intermittently—specifically, 10% of the total operational time with the rate of 4L/min. This intermittent liquid flow was chosen to balance mass transfer efficiency while minimizing potential shear stress on the biofilm. For the initial enrichment phase, the reactors were inoculated with the same microbial consortia and operated exclusively with CO as the sole carbon and energy source. This phase lasted for approximately one month, allowing for

the adaptation and establishment of an active biofilm on the biocarriers. Following this enrichment period, carbon dioxide (CO_2) was introduced into the inlet gas stream alongside CO to assess its impact on microbial activity and conversion efficiency. The gas composition at this stage was BOF adjusted to a $CO/CO_2/N_2$ ratio of 60/20/20(%).

Once the microbial community had adapted to the new gas composition, we systematically increased the gas flow rates in a stepwise manner. The objective was to determine the maximum capacity of each reactor while evaluating key performance indicators such as substrate conversion efficiency, methane yield, and biofilm stability. The results of these performance tests are presented in the following sections. The detailed composition of the inlet gas mixtures and the duration of each experimental sets are summarized in Table 9. These parameters were carefully controlled to ensure consistency across both reactors while allowing for a systematic evaluation of process performance.

Table 9) set of experiments in phase 1

Set	CO (sccm)	N2 (sccm)	H2(sccm)	CO2 (sccm)	Time(d)
1	15	60	0	0	37.22
2	15	5	0	5	14.21
3	20	6.7	0	6.7	10.98
4	25	8.3	0	8.3	2.86
5	30	10	0	10	4.2
6	35	11.7	0	11.7	2.76
7	40	13.3	0	13.3	4.19
8	45	15	0	15	2.82
9	50	16.7	0	16.7	6.08
10	55	18.3	0	18.3	5.15

4.1.1 Impact on Methane Yield and Productivity

The results in the figure 16 illustrate the CO consumption and CH_4 production trends across different experimental phases for both reactors, R1 (RFK packing) and R2 (HF packing).

R1, which is packed with RFK packing, consistently exhibited higher CO consumption (mol/Lpbd) compared to R2 with HF packing. This suggests that the RFK packing may provide a more favorable environment for gas-liquid mass transfer or support a denser or more active biofilm, probably due to the more surface area in R1, that more effectively utilizes CO. Despite this higher substrate uptake in

R1, both reactors produced nearly identical amounts of methane, with R1 showing only a slight improvement in methane output.

This observation indicates that while the rate of CO consumption differs between the reactors, the efficiency of converting CO to methane remains very high and relatively similar in both systems. In fact, the methane yield percentage remained consistently above 90% for both reactors, highlighting the robustness and efficiency of the methanogenic process under the conditions tested. Such a high yield suggests that nearly all of the consumed CO was channeled into methane production, and any variations in CO consumption did not translate into a proportional difference in methane output. This could be due to potential limitations in other factors such as enzyme kinetics or the saturation of microbial metabolic pathways at the operating conditions.

The results of CO consumption percentage in both reactors in the figure 17 indicate that as the flow rates were increased, R2 exhibited a decline in its CO consumption efficiency, eventually reaching a level where only about 90% of the CO was being converted, with approximately 10% exiting the system unconverted. This decrease in CO conversion in R2 suggests that at higher flow rates, the mass transfer and microbial processing capabilities of R2 were being exceeded. Essentially, the increased gas velocity did not allow sufficient residence time or adequate gas-liquid contact, for complete CO uptake, leading to diminishing returns in conversion efficiency. Consequently, further increases in the flow rate for R2 were halted to prevent inefficiencies and potential operational issues.

In contrast, R1, which is packed with RFK material and offers approximately 1.5 times more surface area than R2, maintained a higher percentage of CO consumption even at elevated flow rates. The increased surface area in R1 likely contributed to a denser and more effective biofilm formation, thereby enhancing gas-liquid mass transfer and allowing for more efficient CO conversion. This superior performance underscores the importance of the internal surface area in supporting microbial activity and optimizing reactor performance. The higher efficiency in R1 not only enabled better conversion of the available substrate but also demonstrated the potential benefits of using packing materials that provide enhanced surface area for microbial colonization in syngas biomethanation processes.

In R1, we continued to increase the CO flow rate until we observed a significant decline in performance—specifically, CO consumption dropped to 85% and methane yield began to fall. This decline occurred at a flow rate of 55 SCCM (standard cubic centimeters per minute) of CO, which was more than twice the maximum flow rate previously achieved in R2.

In both reactors, the partial pressure of CO was maintained at approximately 0.9 atm. This relatively high CO partial pressure, while beneficial for providing ample substrate, may also contribute to substrate saturation or inhibitory effects on the microbial consortia when combined with excessively high flow rates. As a result, the overall conversion efficiency and methane yield declined, prompting us to cease further increases in flow rate for R2 and eventually for R1 once similar performance limitations were observed. These findings underscore the critical balance between flow rate, residence time, and gas partial pressure in optimizing syngas biomethanation performance.

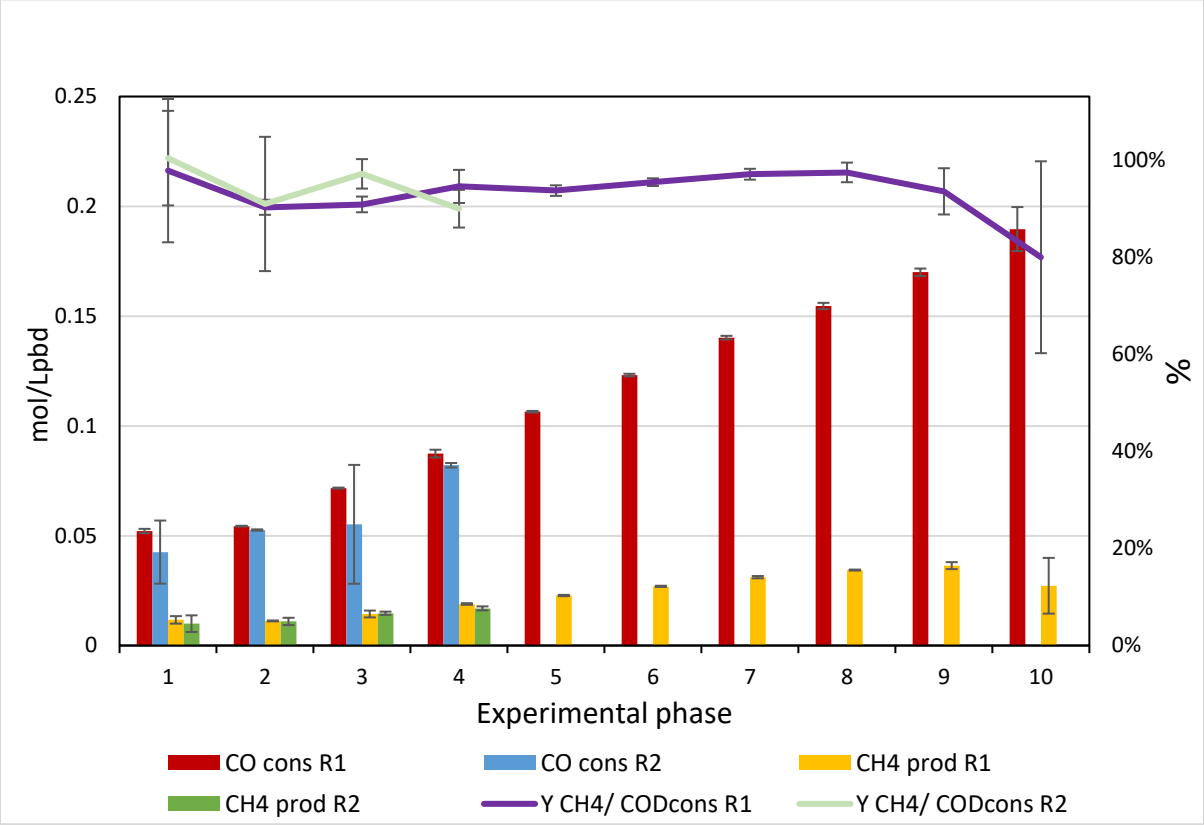


Figure 16) Volumetric methane production, CO consumption and methane production yield of R1 and R2 in phase 1

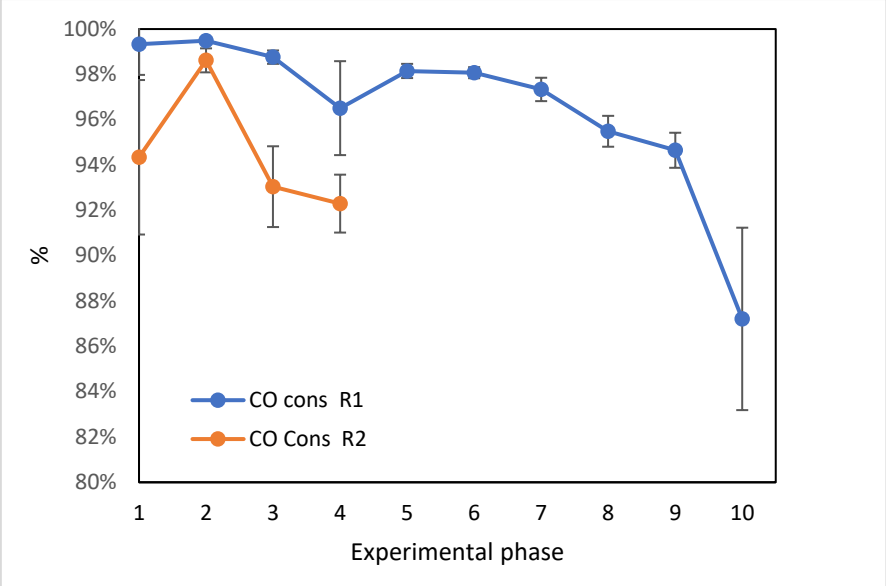


Figure 17) CO consumption percentage for R1 and R2 in phase 1

Figure 18, which displays the pH profiles and VFA concentrations for both reactors, provides valuable insights into the overall stability and efficiency of the methanation process. Notably, the VFAs in both R1 and R2 remain at very low level, indicating that there is no significant accumulation of intermediate acids such as acetate and propionate. This absence of VFA buildup is a strong indicator that the conversion of substrates (e.g., CO, H_2/CO_2) to methane is highly efficient, with the microbial consortia rapidly metabolizing any VFAs as soon as they are produced. Note to mention that literature reports suggest that total VFA concentrations exceeding 2–4 g/L can begin to inhibit methanogenic activity, with severe inhibition typically occurring above 4–6 g/L [88].

Moreover, the stable pH values observed in both reactors suggest that the buffering capacity of the nutrient media, likely enhanced by the phosphate buffer, is effectively maintaining an environment that is conducive to methanogenesis. These findings align well with other performance metrics, such as the high methane yield (over 90%) and the efficient conversion rates observed in both reactors. In essence, the stable pH and negligible VFA levels confirm that the reactors are operating under optimal conditions, with any potential inhibitory effects off acid accumulation being effectively mitigated.

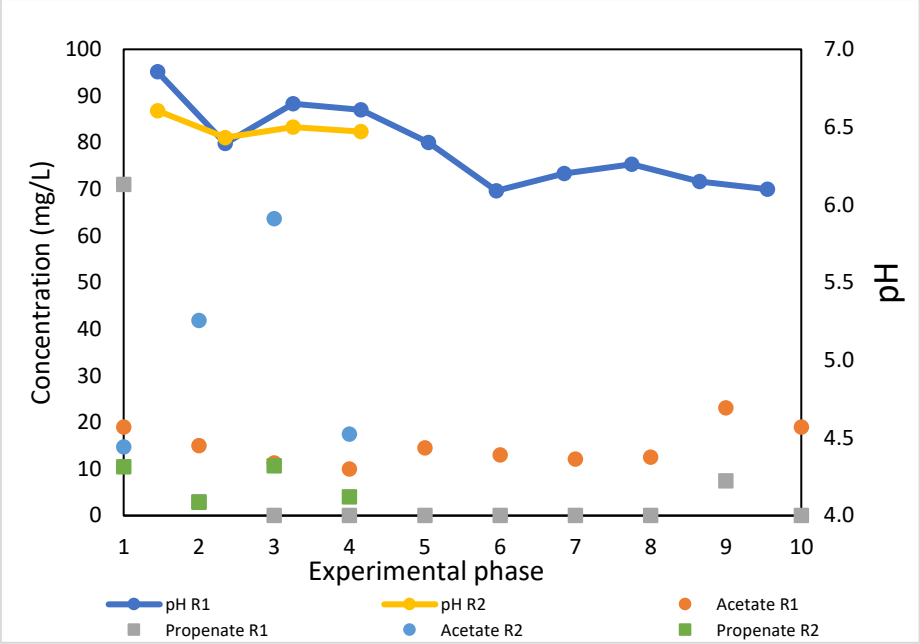


Figure 18) Amount of VFA (namely Acetate and Propionate) and pH for R1 and R2 in phase 1

Figure 19 presented the analysis of VSS (volatile suspended solids) per zone across the reactors from initial sludge and after 117 days of enrichment for both reactors. This revealed marked differences in biomass retention between R1 and R2 over the four-month operating period. In Reactor 1, the bottom zone experienced a slight reduction in VSS, while the middle zone remained essentially unchanged; notably, the top zone showed an approximate 30% increase in VSS. Overall, R1 maintained a stable total VSS, with a slight net growth observed, suggesting effective biofilm retention and even potential biomass accumulation in areas with favorable conditions. In contrast, R2 exhibited a consistent decrease in VSS across all zones, with a clear trend of diminishing biomass from top to bottom.

The observed distribution of biomass, where the top zone exhibits higher VSS compared to the middle and bottom zones in both reactors, can be explained by two primary factors. First, the recirculated liquid media, which is rich in nutrients essential for microbial growth, enters and first contacts the top zone of the reactor. This immediate access to fresh, nutrient-dense media promotes more vigorous microbial proliferation at the top, resulting in greater biomass accumulation compared to the downstream zones. Second, distribution challenges within the reactor may contribute to this pattern. Minor channeling or the development of dry spots between biocarriers could lead to uneven distribution of the liquid media. Such channeling can result in localized areas, particularly in the middle and bottom zones, receiving insufficient nutrient supply. Consequently, these zones would support less microbial growth relative to the top zone.

The overall total VSS in R2 dropped to nearly half of its initial value, indicating a significant failure to maintain the sludge. This reduction in R2 may be attributed to its lower surface area, or suboptimal

conditions for biofilm adhesion, leading to biomass washout or degradation. Consequently, these findings strongly suggest that R2 is unable to sustain the biofilm under the given operational conditions, which is critical for efficient biomethanation.

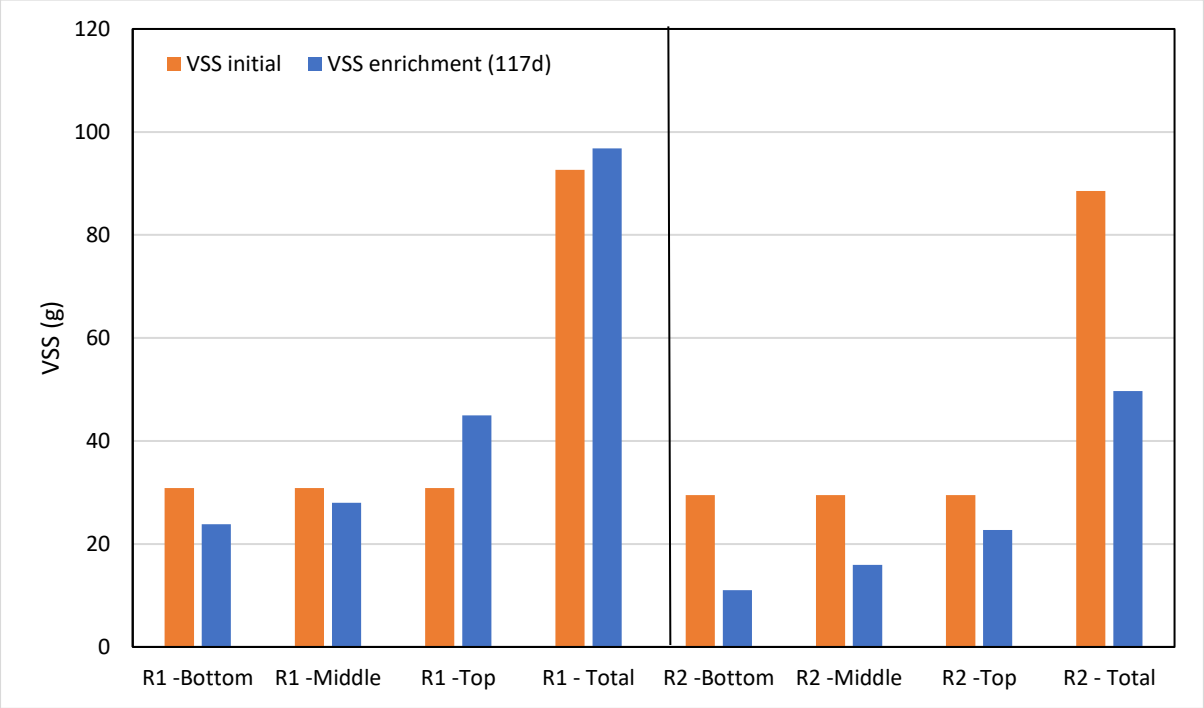


Figure 19) volatile suspended solids per zone across the reactors from initial sludge and after 117 days of enrichment for R1 and R2

Figure 20, the methane production graph, normalized to remove the impact of the surface area and based on COD consumed (g/Lpbd), reveals some important insights when comparing the two reactors. While R1 (equipped with RFK biocarriers) exhibited a slightly higher volumetric COD consumption rate, this did not result in superior methane production when normalized per unit surface area. This observation suggests that although the RFK biocarriers provided a greater specific surface area, the biofilm formation or microbial activity per unit surface was not as efficient. In fact, when evaluating the slopes of the methane production curves ($mol/m^2 \cdot d$ per unit COD consumed), R2 (using HF biocarriers) displays a noticeably steeper slope ($p\text{-value} < 0.05$). This steeper slope indicates that more methane can be produced per m^2 in R2.

The higher rate of COD consumption observed in R2 suggests that the HF biocarriers may facilitate a more rapid conversion rate; however, this apparent advantage is counterbalanced by the limitations imposed by the available COD range and, more critically, by biomass retention. The VSS measurements indicate that R1 maintains a significantly higher amount of biomass compared to R2. Since the biomass (VSS) is the primary driver for methane production in the reactor, the superior biomass retention with RFK biocarriers ultimately results in a more effective and efficient methane conversion process. Thus,

despite the higher substrate consumption rate in R2, the overall performance in terms of methane production is better in R1. This is largely because the RFK biocarriers not only provide a greater surface area for biofilm development but also foster a more robust microbial community, leading to enhanced methane yields. These findings highlight that the increased biomass supported by the RFK biocarriers is the key parameter that drives superior methane production efficiency in R1, making it the better choice for syngas biomethanation under the conditions tested.

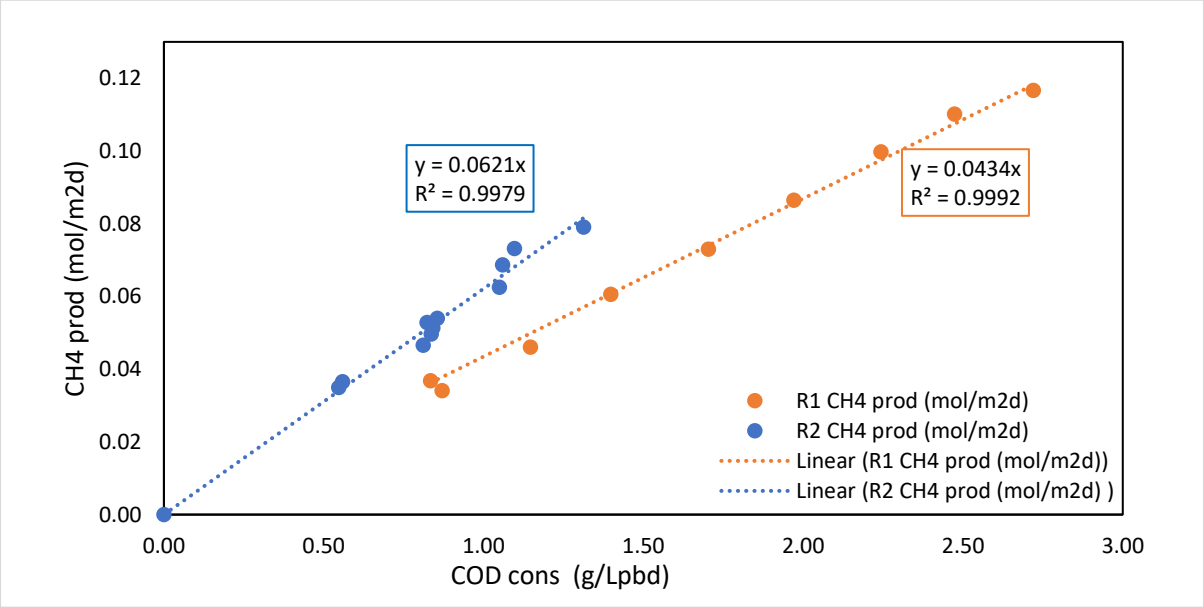


Figure 20) methane production (per surface) based on COD consumed for R1 and R2

4.1.2 Metabolic Activity Test Results

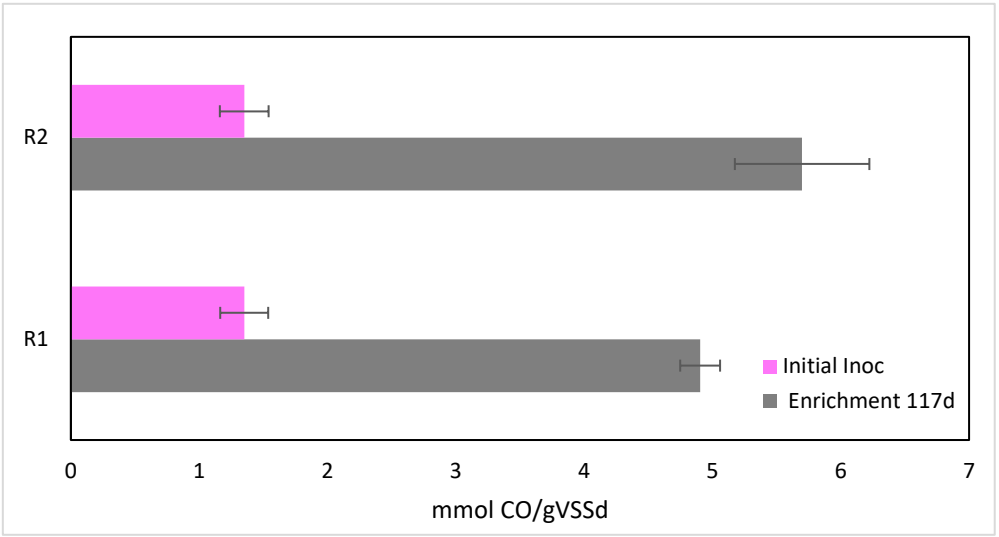
The results of substrate activities are presented in figure 21. The activity tests conducted on CO, H_2, and acetate in both the initial inoculum and after 117 days of reactor operation indicate a clear trend of enhanced metabolic activity over time. In all cases, activities increased after the 117-day enrichment period, suggesting that the microbial communities in both reactors adapted effectively to the operating conditions and substrates provided. However, a notable observation was that R2, which was packed with HF consistently exhibited higher activity levels compared to R1 that utilized RFK packing. The superior activity observed in R2, with notably higher conversion rates for CO, H_2, and acetate, aligns with our previous results demonstrating more efficient methane conversion in R2 compared to R1.

Specifically, the CO conversion activity in R2 increased more than threefold, reaching 6 mmol/gVSSd compared to the initial Lassonde value, while R1 exhibited an activity of 5 mmol/gVSSd. In terms of H_2, the enrichment was notably more pronounced in R2, with activity rising from 70 mmol H_2/gVSSd

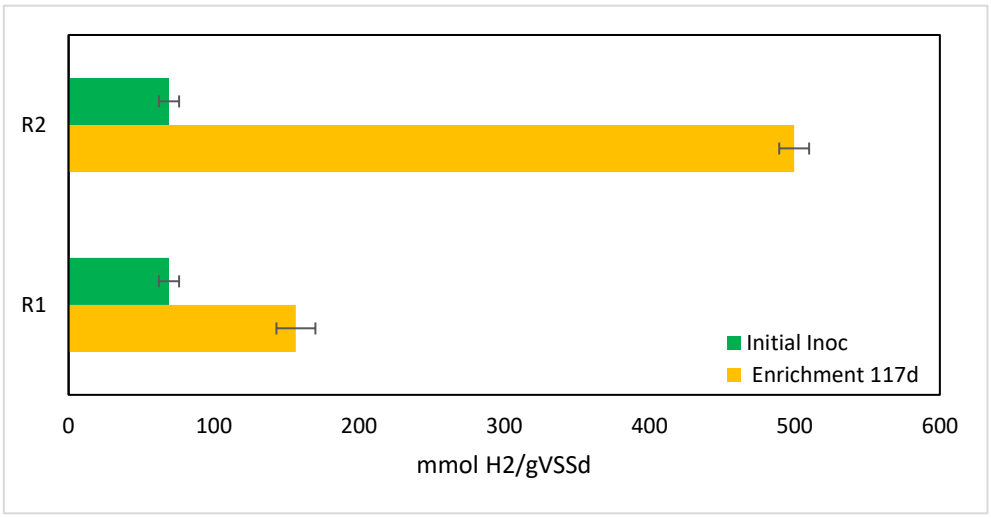
to 500 mmol H_2/gVSSd, whereas R1 achieved 150 mmol H_2/gVSSd. For acetate, activity in R1 remained essentially unchanged, while R2 showed an increase from 3 mmol/gVSSd to 4.5 mmol/gVSS·d.

Furthermore, it has been observed that the Lassonde exhibits a markedly higher activity towards H_2 conversion compared to CO and acetate. This observation suggests that the microbial community within the sludge is particularly enriched with hydrogenotrophic species that preferentially utilize H_2. The significantly elevated H_2 activity could be linked to optimal environmental conditions that enhance hydrogenase enzyme function and promote hydrogen consumption. Additionally, the metabolic pathways predominant in these microbial populations may inherently favor H_2 utilization over CO and acetate, which will be investigated via metagenome study further.

A)



B)



C)

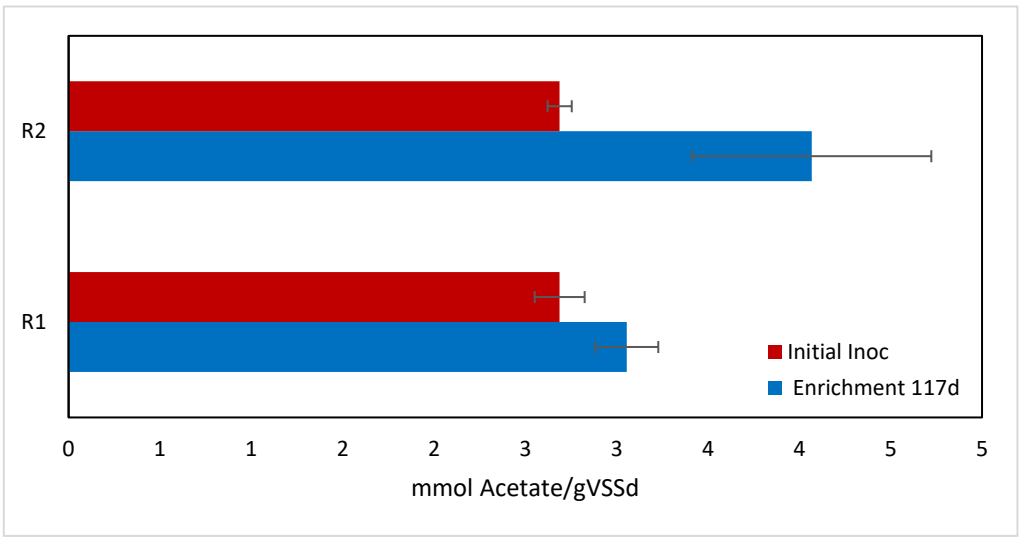


Figure 21) A) CO, B) H2 and C) Acetate activity results of the initial inoculum compare to 117 days of experiment for R1 and R2

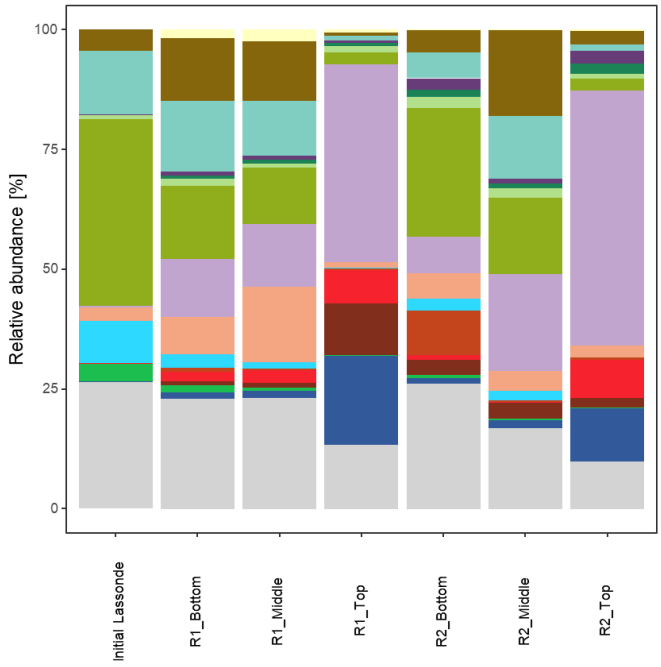
4.1.3 Metagenomic Insights for Phase 1

In the initial metagenomic analysis for Phase 1 in figure 22, the relative abundance profiles of microbial populations in the initial sludge were compared with those in the top, middle, and bottom zones of the biofilm in both R1 and R2. Overall, the microbial communities across both reactors exhibited almost similar relative abundances, indicating that the initial inoculum colonized the biocarriers in R1 and R2 comparably. This similarity suggests that both reactors provided analogous environments for microbial growth during the initial colonization phase.

A notable exception was observed in the top zones of the reactors. While the microbial community structure in the middle and bottom zones remained consistent between R1 and R2, the top zones showed differences that can likely be attributed to a foaming phenomenon witnessed during the early stages of inoculation. In the initial weeks, the reactor vessels experienced significant foaming—more intense in R2—likely comprised of dead cells and loosely attached biomass. This foaming effect appears to have disrupted the typical biomass attachment, effectively transporting non-adherent cells to the top zones of the biofilm.

Within the community the methanogenic species were represented by two archaea phyla: *Halobacteriota* and *Methanobacteriota*, However, only the last one appeared to play a dominant role. In particular, the relative abundance of *Methanobacterium formicicum* was found to be higher in the top zones of both reactors (approximately 50%). This enrichment is plausibly linked to the aforementioned foaming, where the biomass, including *M. formicicum*, was mobilized and accumulated at the reactor tops. Such a spatial redistribution of key methanogens due to physical disturbances underscores the impact that operational phenomena like foaming can have on the overall microbial community structure and, potentially, on reactor performance.

a)



Taxonomy

- d__Archaea;p__Halobacteriota;c__Methanomicrobia;o__Methanomicrobiales;
- d__Archaea;p__Halobacteriota;c__Methanosarcinia;o__Methanotrichales;f__Methanotrichaceae;g__Methanothrix;s__Methanothrix soehngenii (bin.101)
- d__Archaea;p__Halobacteriota;c__Methanosarcinia;o__Methanotrichales;f__Methanotrichaceae;g__Methanothrix;s__Methanothrix sp002067755 (bin.113)
- d__Archaea;p__Methanobacteriota;c__Methanobacteria;o__Methanobacteriales;
- d__Archaea;p__Methanobacteriota;c__Methanobacteria;o__Methanobacteriales;f__Methanobacteriaceae;
- d__Archaea;p__Methanobacteriota;c__Methanobacteria;o__Methanobacteriales;f__Methanobacteriaceae;g__Methanobacterium_A;s__ (bin.288)
- d__Archaea;p__Methanobacteriota;c__Methanobacteria;o__Methanobacteriales;f__Methanobacteriaceae;g__Methanobacterium_A;s__Methanobacterium_A sp002
- d__Archaea;p__Methanobacteriota;c__Methanobacteria;o__Methanobacteriales;f__Methanobacteriaceae;g__Methanobacterium;s__Methanobacterium formicicum
- d__Archaea;p__Methanobacteriota;c__Methanobacteria;o__Methanobacteriales;f__Methanobacteriaceae;g__Methanobacterium;s__Methanobacterium sp0020670
- d__Bacteria;p__Actinomycetota;c__Actinomycetes;o__Propionibacteriales;f__Propionibacteriaceae;g__Brooklawnia;s__Brooklawnia sp012517405 (bin.36)
- d__Bacteria;p__Bacillota_A;c__Clostridia;o__Eubacteriales;f__Eubacteriaceae;g__Acetobacterium;s__Acetobacterium wieringae (bin.29)
- d__Bacteria;p__Bacillota_B;c__Thermincolia;o__Thermincolales;f__UBA2595;g__;s__ (bin.38)
- d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Tidjanibacter;s__ (bin.81)
- d__Bacteria;p__Chloroflexota;c__Anaerolineae;o__Anaerolineales;f__Anaerolineaceae;g__T78;
- d__Bacteria;p__Synergistota;c__Synergistia;o__Synergistales;f__Synergistaceae;g__Syner-03;s__Syner-03 sp002306075 (bin.235)
- Other

b)

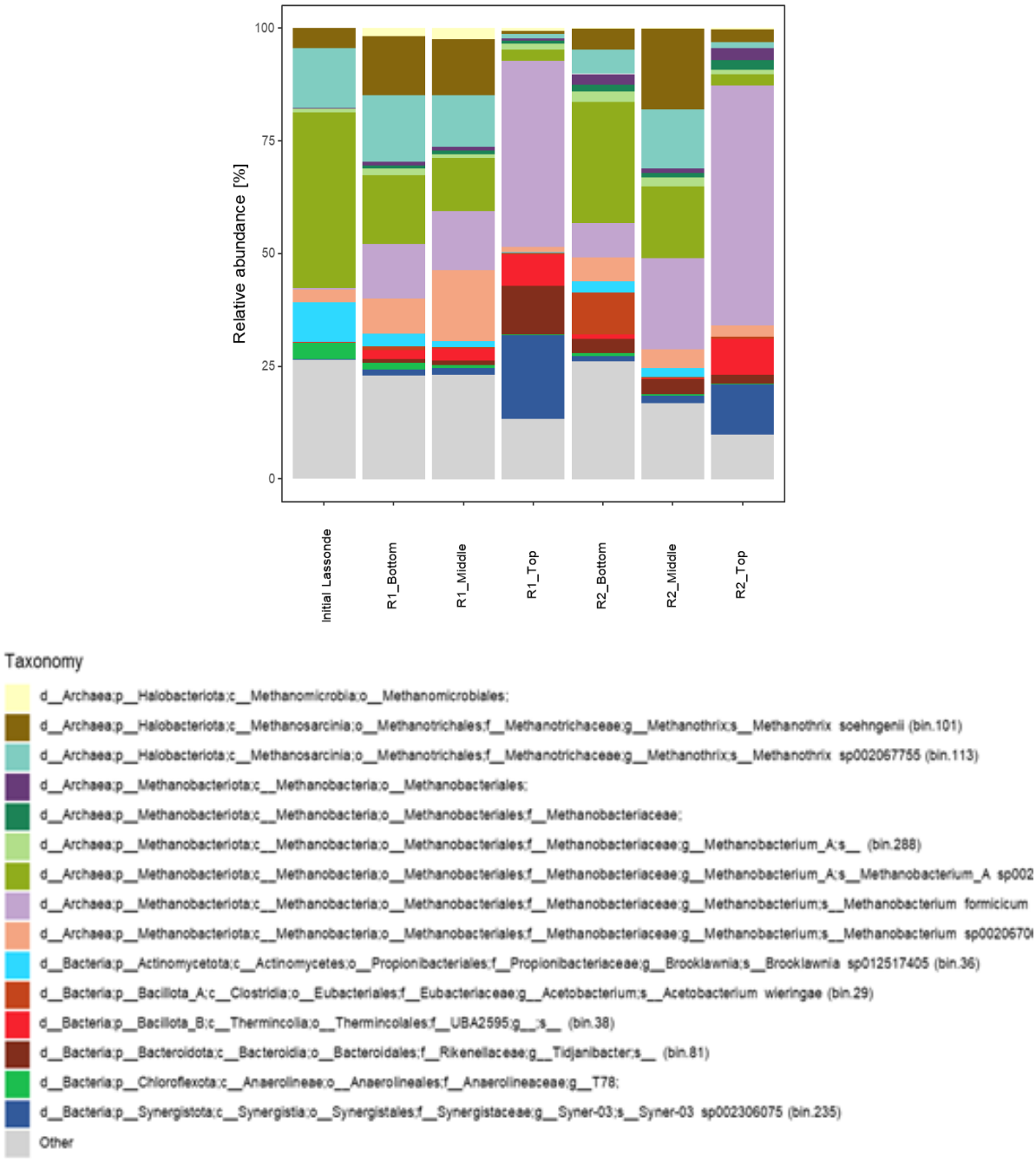


Figure 22) Relative abundance of microbial communities of initial sludge compare to biofilm in bottom, middle, top zone of a) R1 and b) R2 in phase 1

In the figure 23, showed the profile of the microbial communities of biomass in the liquid throughout the days of the experiments. After one month of operation, the microbial community in both reactors appeared to have reached a state of relative stabilization. In particular, R2 demonstrated a pronounced stabilization, with *Methanobacterium formicicum* accounting for nearly 70% of the microbial taxa. This dominant presence indicates that the conditions within R2, likely influenced by its biocarrier design and operating parameters, selectively favored the proliferation of this key

methanogen. Such a high relative abundance suggests that *M. formicicum* played a central role in syngas biomethanation under the conditions tested, contributing significantly to the overall methane production efficiency. In contrast, R1 exhibited a more dynamic microbial community profile over the same period. Although stabilization occurred, the community in R1 evolved to become more diverse over the long term, as evidenced by a broader range of taxa present after prolonged exposure to syngas in continuous mode. The evolving microbial landscape in R1 could potentially enhance the resilience of the system to operational fluctuations, though it might also imply a less specialized pathway for methane production compared to R2.

Overall, these findings underscore the influence of the reactor configuration and operational mode on the structure and dynamics of microbial communities in syngas biomethanation. While R2's rapid stabilization and dominance of M. formicicum point to an efficient, specialized conversion process, the more diverse community in R1 suggests an alternative strategy that might benefit system stability and adaptability.

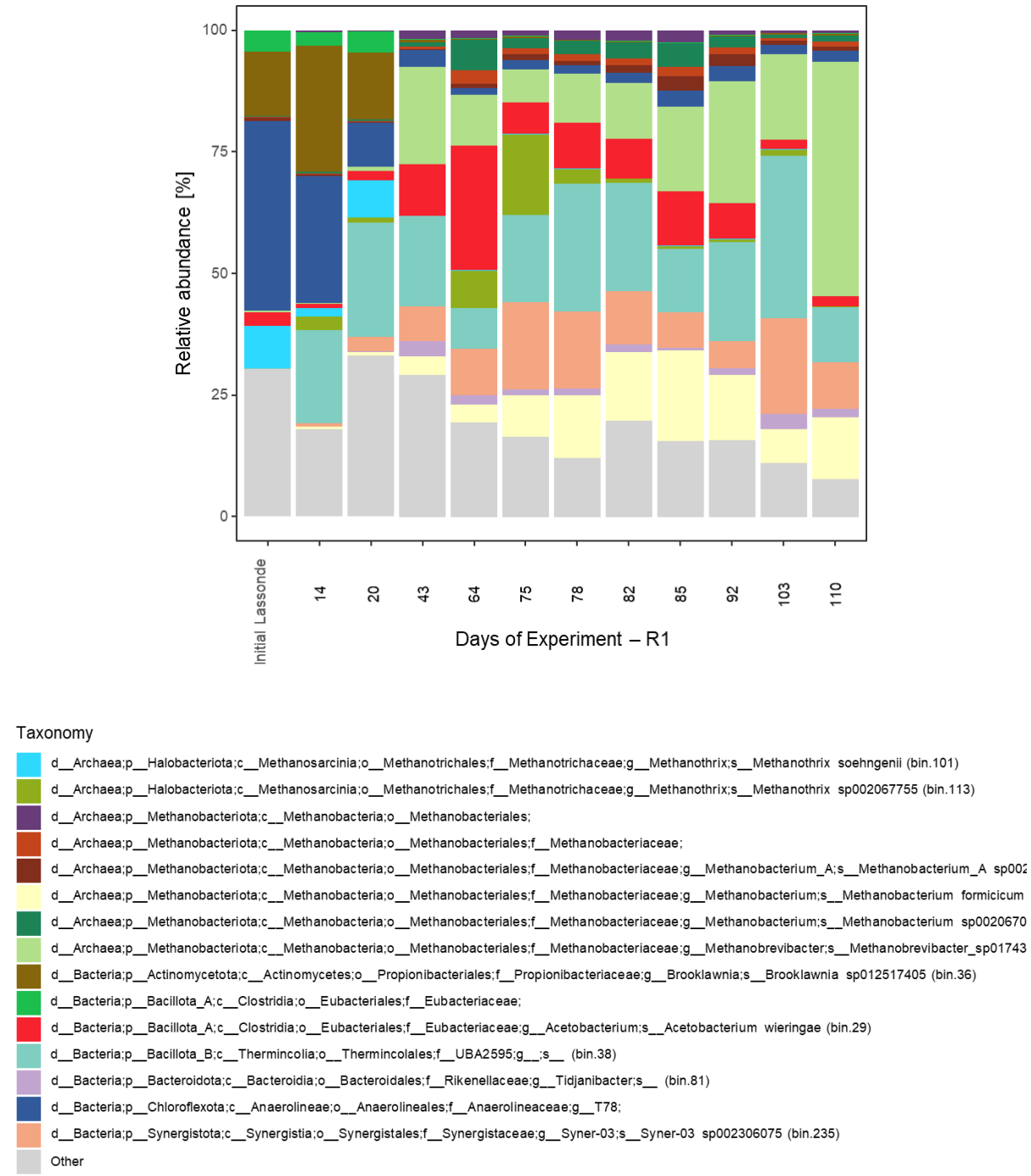


Figure 23) Relative abundance of microbial community of initial sludge compared to liquid in days of experiment of R1 and R2 in phase 1

Analysis of the microbial community composition reveals a marked difference between the two reactors throughout the days of experiment by changing syngas composition. In R1, less than 50% of the microbial population consisted of methanogenic archaea (primarily *Methanobacteria*), while *Thermincolales* bacteria accounted for approximately 20–30% of the community. In contrast, R2 was dominated by archaea—nearly 70% of its microbial population—with a lower proportion of bacteria. This disparity in community composition has important implications for biomethanation performance.

The methanogenic archaea, particularly species within the *Methanobacteria*, play a central role in converting substrates such as H_2 and CO_2 into methane via hydrogenotrophic methanogenesis [89]. The high relative abundance of these archaea in R2 likely contributes directly to its more efficient methane production, as a greater proportion of the biomass is dedicated to this key conversion process. In R1, the lower abundance of methanogens, coupled with a higher proportion of *Thermincolales* bacteria, suggests a more complex or competitive microbial ecosystem. *Thermincolales* have been associated with the degradation of complex organic substrates and might compete for intermediates that would otherwise be utilized by methanogens [90].

4.1.5 Discussion and Implications

Based on the results obtained from Phase 1, RFK packing was identified as the superior biocarrier compared to HF. The primary factor contributing to this conclusion is RFK's enhanced ability to retain biomass, as evidenced by the higher volatile suspended solids (VSS) observed in reactors utilizing RFK. This superior biomass retention is crucial because it directly influences the reactor's capacity for methane production. The greater VSS content indicates a denser and more robust microbial community, which facilitates more efficient biomethanation.

The key differentiator between RFK and HF lies in the structural properties of the biocarriers. RFK provides a significantly larger surface area, which promotes the development of thicker, more stable biofilms. This extensive surface area not only supports higher microbial loading but also enhances the spatial distribution of methanogenic archaea, creating optimal microenvironments for their metabolic activities. The biofilm structure in RFK allows for better mass transfer of gases like CO and H_2, improving substrate availability to the microorganisms, which ultimately leads to higher methane yields [91].

Furthermore, the physical characteristics of RFK—being lighter in weight and made from cheaper recycled materials—offer additional economic and operational advantages. The reduced weight simplifies reactor handling and installation, while the cost-effectiveness of recycled materials makes RFK a more sustainable choice for large-scale applications. This economic benefit does not compromise performance; instead, it complements RFK's biological efficiency, making it a practical and high-performing biocarrier for syngas biomethanation. While HF demonstrated a higher activity rate in some cases, particularly in the early stages of enrichment, this did not translate into superior long-term methane production. The limited biomass retention capability of HF likely hindered the establishment of a stable and productive microbial community, which is critical for continuous operation. Additionally, the observed foaming phenomena in HF-packed reactors further disrupted biofilm stability, contributing to inconsistent performance.

In conclusion, the combination of enhanced biomass retention, superior surface area for biofilm development, and favorable economic factors firmly establish RFK as the more effective and sustainable biocarrier for biomethanation processes under the conditions tested. These findings have significant implications for the design and optimization of bioreactors in industrial applications, highlighting the importance of selecting biocarriers that support both biological performance and operational efficiency.

4.2 Phase 2: Effect of Pressure

In this phase, we investigated the impact of pressure variations on the biomethanation process. Similar to the previous phase, a detailed set of experiments was conducted for both reactors, and the experimental parameters are summarized in Table 10.

Hence, both reactors were inoculated with the same selected biocarrier material (RFK) to ensure that any observed differences in performance could be attributed to the operating pressure rather than the type of packing. The reactors were subjected to two distinct operating pressures: 1.5 atm and 2.5 atm. These pressure conditions were chosen to assess how increased pressure would influence reactor performance, particularly in terms of CO and H_2 consumption, methane production, and overall process efficiency. The aim was to evaluate whether higher pressure could enhance the methanation process by facilitating better gas-liquid mass transfer and by promoting more optimal microbial activity within the system. The subsequent analysis compares the results from both pressure conditions, focusing on key performance metrics such as CO and H_2 consumption rates and methane yield, highlighting any significant impact of pressure variation on reactor behavior and efficiency. The Inlet gas composition started with BFG (of 8% H_2, 44% N_2, 25% CO, and 23% CO_2).

By comparing the performance at 1.5 atm and 2.5 atm, we sought to determine the optimum pressure range that maximizes substrate conversion while ensuring stable reactor operation. The higher-pressure condition was carefully selected based on safety criteria and to explore the potential for enhanced mass transfer between the gas and liquid phases.

Table 10) set of experiments in phase 2

Set	CO (sccm)	N2 (sccm)	H2 (sccm)	CO2 (sccm)	Time(d)
1	10	17.6	3.2	9.2	6.05
2	20	34.8	6.4	18.4	8.84
3	30	52.7	9.6	27.6	5.91
4	35	61.6	11.2	32.2	7.02
5	40	70.4	12.8	36.8	4.99
6	45	79.2	14.4	41.4	7.01
7	50	88	16	46	7.01
8	55	96.8	17.6	50.6	6.99
9	50.9	89.6	32.6	46.9	7.88
10	60	105.6	38.4	55.2	2.09
11	50.9	89.6	32.6	46.9	3.93
12	0	0	176	44	2.3
13	0	110	88	22	7.7
14	40	70.4	12.8	36.8	3.03
15	20	34.8	12.8	18.4	5.95

4.2.1 Methane Yield under Different Pressures

Figure 24 illustrates the performance of R1 and R2 in different experimental phases. The results show that both reactors—operating at 1.5 atm (R1) and 2.5 atm (R2) under with a BFG feed (8% H_2, 44% N_2, 25% CO, and 23% CO_2) delivered methane yields above 90% up to phase 7 when the gas flowrates were increased gradually. However, in phase 8 the reactor at higher pressure (R2) experienced a drop in yield to about 82%. A close examination of figure 25 indicates that this reduction coincides with a pH decline in R2, which fell to approximately 6.1 with increasing gas flowrates.

Maintaining an optimal pH is critical for efficient biomethanation because methanogenic archaea, particularly hydrogenotrophic types that consume H_2 and CO_2, are very sensitive to acidic conditions. Most methanogens have an optimum pH range near neutrality (approximately 6.8–7.2) and their enzymatic machinery is designed to operate efficiently only above pH 6. When the pH drops below 6, several adverse effects occur. First, the activity of key methanogenic enzymes is inhibited, slowing the conversion of substrates into methane. Second, a low pH can alter the integrity of microbial cell membranes and disturb intracellular pH homeostasis, further hampering the metabolic processes needed for methane formation. Taconi et al. [92] reported that methanogenesis is significantly

inhibited when the pH falls below 6, leading to reduced substrate consumption and lower methane yields.

Moreover, the COD consumption data on figure 24 supports these findings: In R2, the high pressure likely exacerbated issues related to the mass transfer, leading to incomplete utilization of the available COD. On the other hand, R1's lower operating pressure allowed for a more balanced conversion process, maintaining high methane yields despite a lower rate of COD consumption. These results emphasize that while higher pressure can enhance gas solubility, it must be carefully balanced with flow rate and reactor design to prevent limitations in mass transfer and substrate utilization.

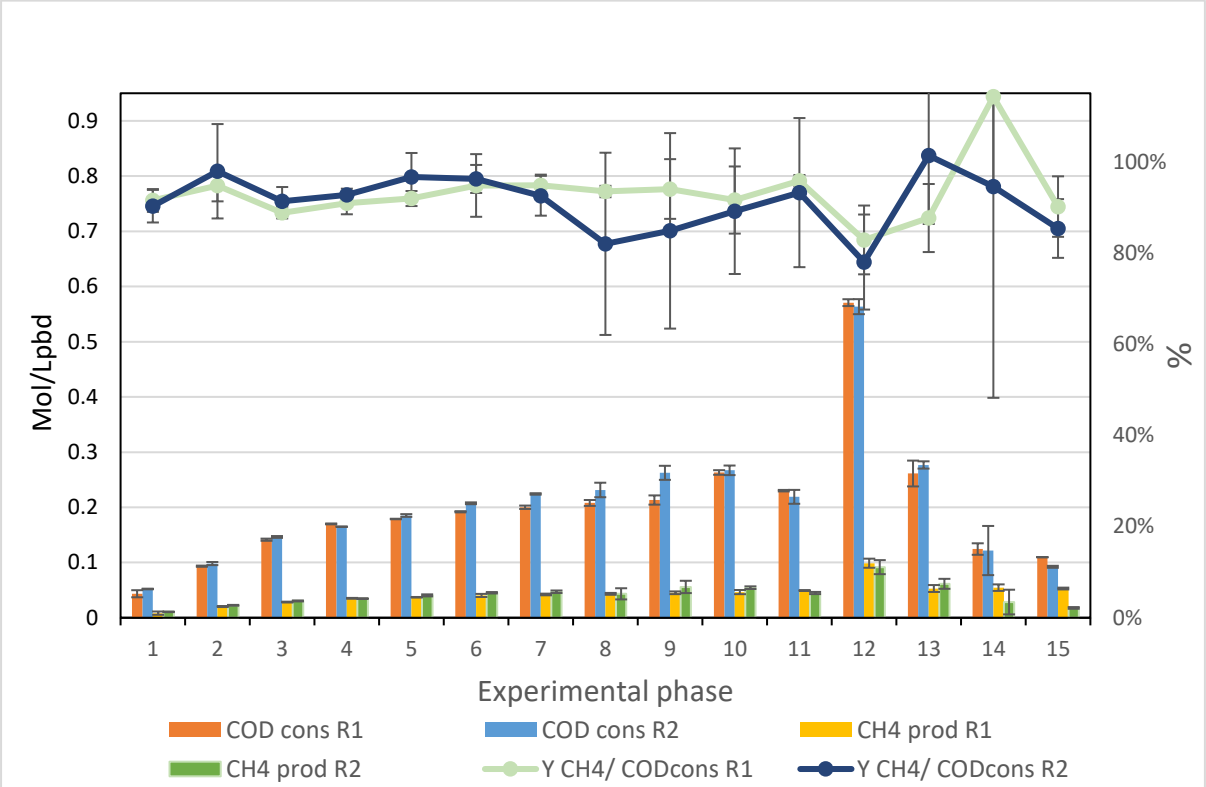


Figure 24) Volumetric methane production, CO consumption and methane production yield of R1 and R2 in phase 2

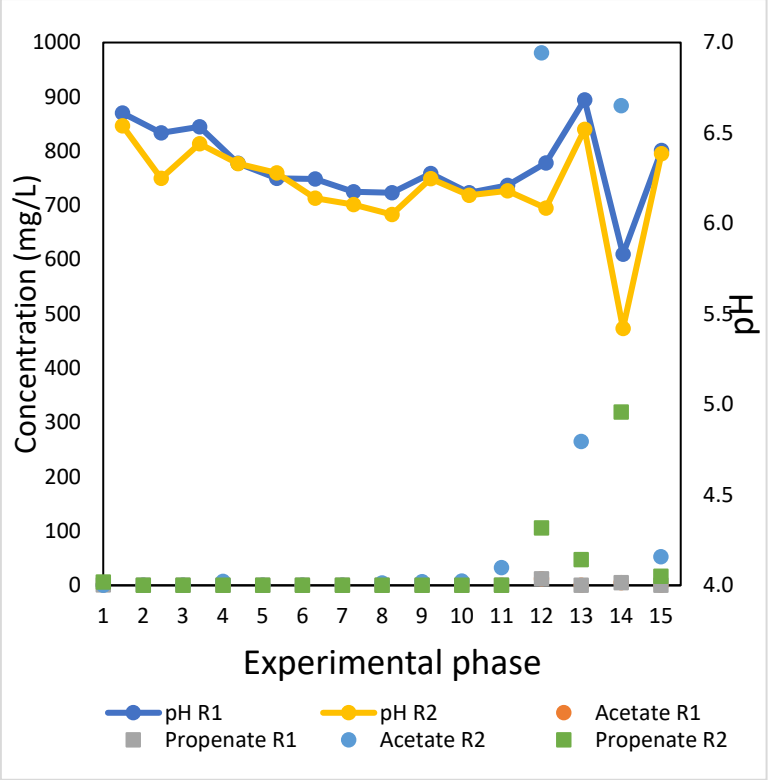


Figure 25) Amount of VFA (namely Acetate and Propionate) and pH for R1 and R2 in phase 2

Moreover, figure 25 shows that while the consumption of CO remains largely similar in both reactors across the phases, a marked drop in the H_2 consumption is observed in phase 8 for R2. In this phase, about 15% of the H_2 remains unconsumed. Since hydrogenotrophic methanogens rely on H_2 as the electron donor for converting CO_2 into CH_4, the incomplete uptake of H_2 directly points to the suppression of their activity. This further supports the interpretation that the pH drop in R2 is adversely affecting the hydrogenotrophic pathway. In a higher pressure reactor, a larger fraction of H_2 (CO and CO_2) dissolves in the liquid phase, according to Henry's law; while this can be beneficial for reaction rates, it also means that any pH shifts induced by changes in dissolved inorganic carbon or acid–base equilibria may be more pronounced. In R2, the intensified dissolution appears to have led to a more significant pH drop, thereby inhibiting the methanogenic population.

Based on the data presented in figure 26, the concentration of volatile fatty acids (VFAs) in both reactors operating under the BFG gas inlet conditions was essentially zero.

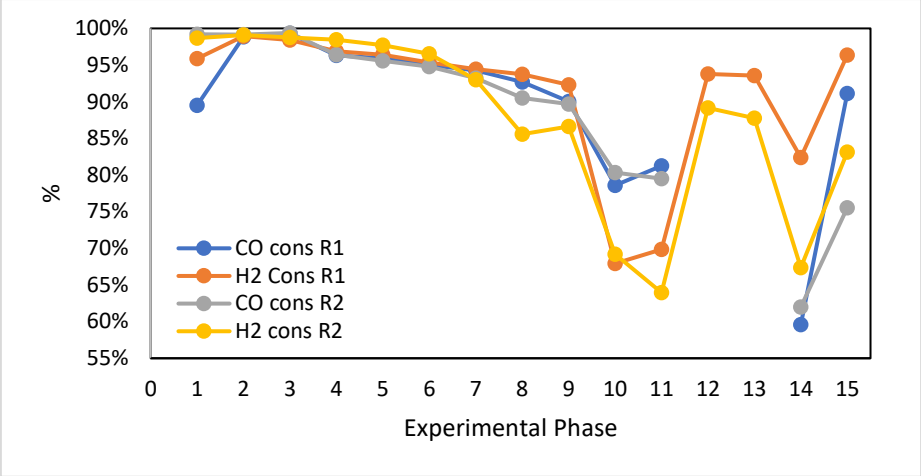


Figure 26) CO and h_2 consumption percentage for R1 and R2 in phase 2

In phases 9–11 the strategy of increasing the H_2 fraction in the inlet gas helped to recover the dropped pH in the reactor R2, and this adjustment corresponded with an increased methane yield. The increased H_2 not only promoted a more favorable buffering capacity but also ensured that hydrogenotrophic methanogens had a sufficient electron donor for converting CO_2 into CH_4 [93]. However, in phase 10 the gas inlet flow rate—with its higher H_2 percentage—appeared to exceed the metabolic capacity of the biomass. As a result, the consumption efficiencies of CO and, more markedly, H_2 dropped to approximately 80% and 65%, respectively. Such a decline in substrate consumption under excessive H_2 conditions is consistent with previous findings that both insufficient and excessive H_2 supply can disturb mass transfer and enzyme kinetics in anaerobic systems [94]. This study emphasizes that while a higher H_2 supply can be used to counteract pH drops (through increased dissolution of CO_2 and its conversion into buffering species), an overload can lead to mass transfer limitations and inhibitory effects that reduce the overall efficiency of the biomethanation process.

In phases 12 and 13 of the experiment, the gas composition was changed to an H_2/CO_2 mixture. Figure 26 shows that while reactor R1 (operating at a lower pressure) consumed 95% of the supplied H_2, reactor R2 (operating at higher pressure) exhibited a lower H_2 consumption efficiency with about 10% of the H_2 remaining unconsumed, although the total methane yield of both reactors was almost identical (figure 24). This difference suggests that high-pressure conditions can overstimulate the dissolved H_2 concentration, thereby pushing the available H_2 beyond the metabolic capacity of the biomass. In other words, the high-pressure reactor is more sensitive to excessive H_2, which can cause mass transfer limitations and enzyme saturation, ultimately leading to lower conversion efficiencies.

In these phases, R2 exhibits a substantial increase in VFA amount, namely 981 mg/L Acetate and 100 mg/L of Propionate. When the microbial community is exposed to an H_2 supply that exceeds its processing ability, the rate of hydrogen consumption declines (as seen by the 10% unconsumed H_2 in

R2) and the subsequent conversion of intermediate products (such as VFAs) is inhibited. In contrast, the lower pressure reactor R1 is less prone to such overload, enabling it to achieve near-complete H_2 consumption and efficient conversion of VFAs into methane. It is important to note that this H_2 consumption issue was not due to CO intoxication, as CO was absent from the gas mixture during these phases. This eliminates CO-related inhibition as a contributing factor, further supporting the hypothesis that the observed inefficiency is primarily driven by pressure-induced mass transfer and metabolic constraints.

In phases 14 and 15 the reactors were switched back to feeding BGF with a higher H_2 proportion in an attempt to prevent pH drop. Despite the intention, both reactors experienced a decrease in pH; however, reactor R2 exhibited a more pronounced pH decline (down to approximately 5.4) compared to R1 (which dropped to about 5.8). This further led to a significant reduction in both CO and H_2 consumption rates in both reactors, with R2 showing the most dramatic decrease.

Consequently, as shown in figure 24, the methane production in R2 was roughly half that of R1 (0.053 mol/Lpbd in R1 compared to 0.018mol/Lpbd). These results indicate that excessive H_2 - especially under conditions where the system is already stressed by high pressure - can lead to a pH drop that likely inhibits key methanogenic pathways. When the biomass becomes overloaded with H_2, the ensuing pH drop can inhibit the hydrogenotrophic methanogenic pathway, as well as the syntrophic relationships required for complete VFA conversion. In our experiment, the lower pH in R2 (5.4) compared to R1 (5.8) is a clear indicator that the excess H_2 - rather than providing more substrate - triggered inhibitory conditions that compromised the efficiency of the methanogenic community. This correlation between pH, substrate consumption, and methane production underscores the critical importance of balancing hydrogen dosing, particularly under high-pressure conditions where even slight deviations in pH can lead to substantial performance losses.

Figure 27 presented the analysis of VSS per zone across the reactors from initial sludge and after enrichment for both reactors. The results indicate that, after enrichment, the VSS levels have increased in all zones of both reactors, confirming that the microbial biomass has proliferated during the process in TBR. Notably, the bottom zone of reactor R2 shows a significantly higher VSS concentration compared to reactor R1. Since the reactor is fed from the bottom, the fresh substrate is introduced directly at the lowest level with higher partial pressure. In reactor R2—where the operating pressure is higher—the hydrostatic pressure at the bottom is even more pronounced. This elevated pressure likely enhances local mass transfer and improves biomass retention by reducing washout, thereby fostering the formation of denser microbial aggregates right at the feed inlet. Consequently, the conditions at the bottom zone become particularly favorable for microbial growth,

leading to a greater increase in VSS compared to the upper zones or to reactors operating at lower pressure. These observations underscore the crucial influence of feed positioning and pressure on reactor performance.

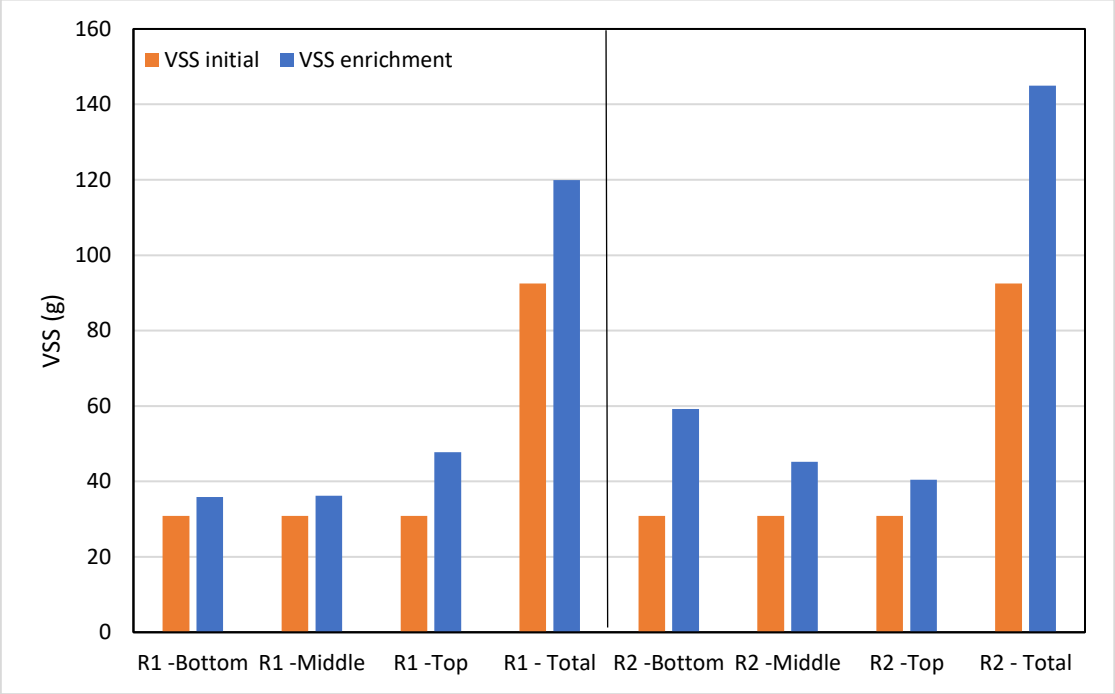


Figure 27) VSS per zone across the reactors from initial sludge and after enrichment for R1 and R2 in phase 2

4.2.2 Metagenomic Insights of phase 2

In the initial metagenomic analysis for Phase 2 in figure 28, the relative abundance profiles of microbial populations in the initial sludge were compared with those in the top, middle, and bottom zones of the biofilm in both R1 and R2. Microbial composition differences between corresponding sampling points of R1 and R2 were modest. This suggests that the different pressure levels were not considerably affecting the composition of microbiomes. Therefore, the higher solubility of gasses induced in R2 did not select species for particular specific metabolic adaptation. These findings are consistent with the results reported by Ebrahimian et al. [59].

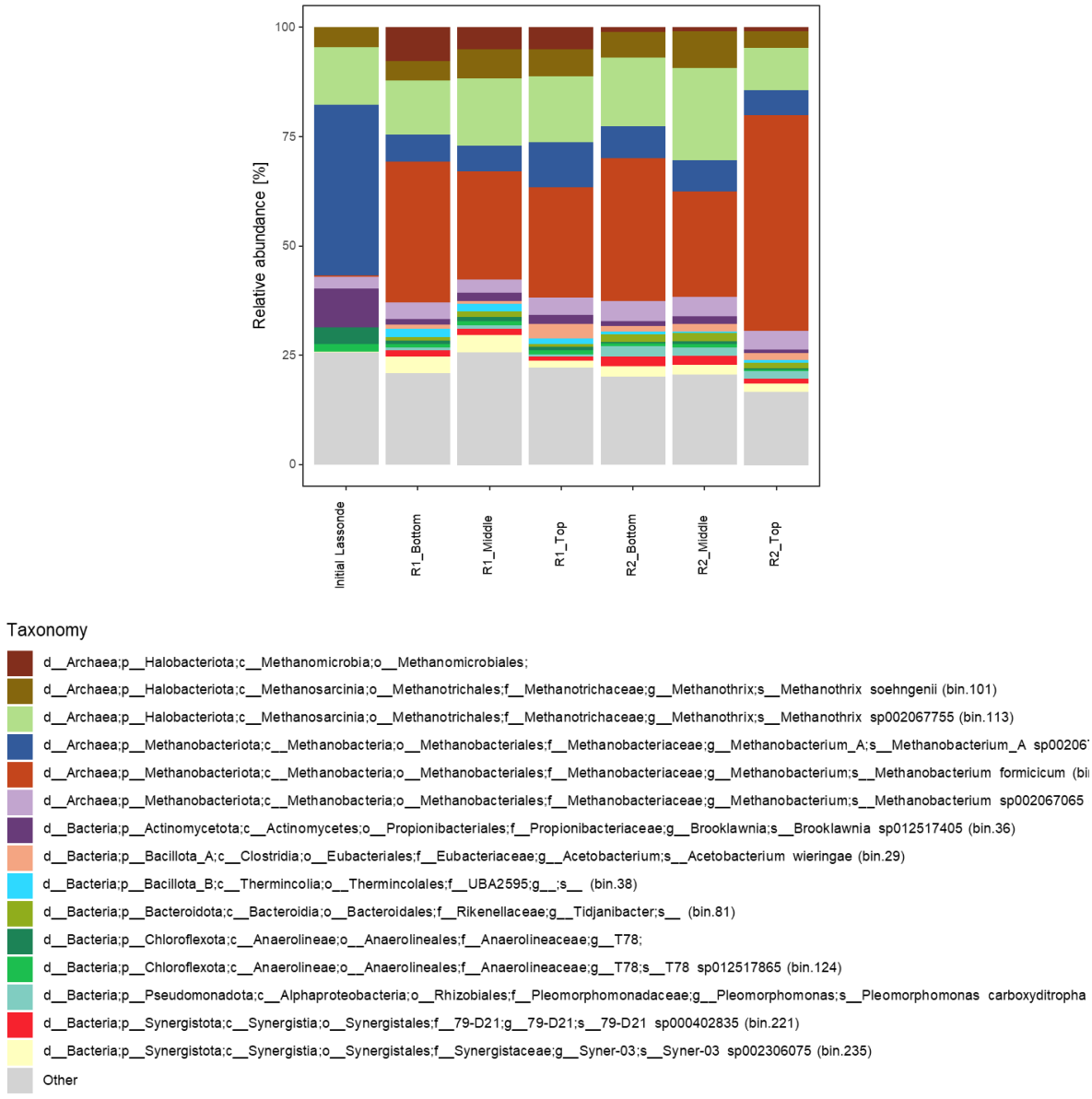


Figure 28) Relative abundance of microbial communities of initial sludge compare to biofilm in bottom, middle, top zone of R1 and R2 in phase 2

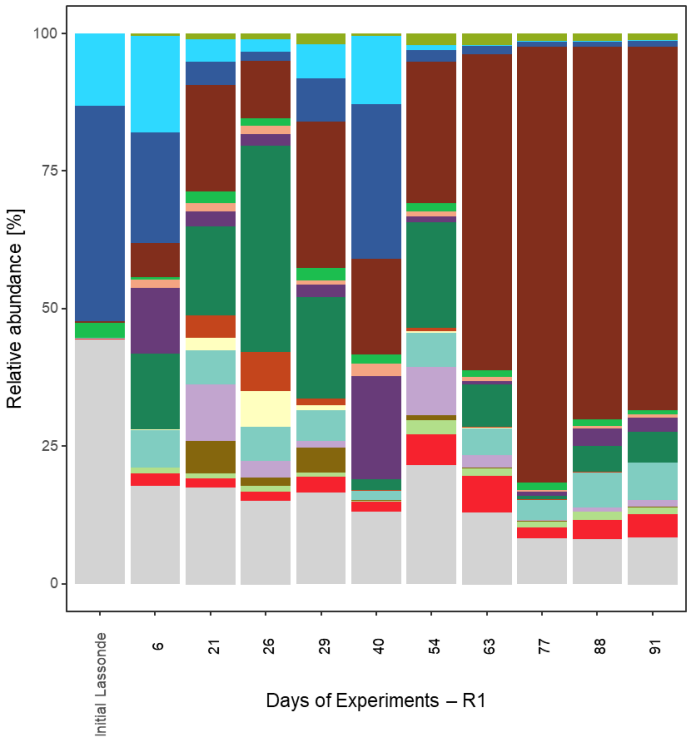
The metagenomic results from phase 2 (Figure 28) clearly show that *Methanobacterium formicicum* dominates the biofilm in both R1 and R2 across all zones. *Methanobacterium formicicum* is a well-characterized hydrogenotrophic methanogen known for its ability to efficiently utilize H_2 as an electron donor and CO_2 as a carbon source [95]. The consistent dominance of *M. formicicum* in all zones of the biofilm suggests that it can establish itself regardless of localized variations in nutrient gradients or pH. Such uniformity implies that it plays a pivotal role in maintaining stable methanogenic activity across the reactor, ensuring a reliable conversion of H_2 and CO_2 to methane.

Figure 29 illustrated the relative abundance of microbial communities of liquid media of reactors throughout the days of the experiments. Based on the metagenomic analysis of the liquid media

throughout the experiments, it was found that in reactor R1 (especially while feeding with BFG gas), the relative abundance of *Terminocolales* bacteria was in the range of 20–30%, whereas in reactor R2 the community was consistently dominated by *Methanobacterium*. This divergence in community composition can be interpreted as a reflection of how operational conditions - such as gas composition, pressure, and hydrogen partial pressure - shape the microbial consortia in anaerobic systems. Recent studies have shown that microbial community structure in anaerobic digesters is highly sensitive to hydrogen availability and reactor operating pressure. For example, in an integrated biogas upgrading system, Luo and Angelidaki [96] demonstrated that under moderate hydrogen partial pressures, the community remains relatively diverse and includes significant populations of fermentative or syntrophic bacteria. These bacteria (which could belong to groups such as Termiticolales) are actively involved in the breakdown of complex substrates, such as extracellular polymeric substances (EPS), cell lysis products (proteins, lipids), and longer-chain volatile fatty acids (e.g., propionate and butyrate), facilitating interspecies electron transfer and helping maintain a balanced conversion of substrates to methane.

On the other hand, when conditions favor a high dissolved hydrogen concentration—often as a consequence of elevated pressure—the hydrogenotrophic methanogens (such as *Methanobacterium*) are competitively favored because of their high-affinity hydrogenases and their capacity to efficiently convert H_2 and CO_2 into methane.

a)



Taxonomy

- d__Archaea;p__Halobacteriota;c__Methanomicrobia;o__Methanomicrobiales;
- d__Archaea;p__Halobacteriota;c__Methanosarcinia;o__Methanotrichales;f__Methanotrichaceae;g__Methanothrix;s__Methanothrix sp002067755 (bin.113)
- d__Archaea;p__Methanobacteriota;c__Methanobacteria;o__Methanobacteriales;f__Methanobacteriaceae;g__Methanobacterium_A;s__Methanobacterium_A sp002
- d__Archaea;p__Methanobacteriota;c__Methanobacteria;o__Methanobacteriales;f__Methanobacteriaceae;g__Methanobacterium;s__Methanobacterium formicicum
- d__Archaea;p__Methanobacteriota;c__Methanobacteria;o__Methanobacteriales;f__Methanobacteriaceae;g__Methanobacterium;s__Methanobacterium sp0020670
- d__Bacteria;p__Bacillota_A;c__Clostridia;o__Eubacteriales;
- d__Bacteria;p__Bacillota_A;c__Clostridia;o__Eubacteriales;f__Eubacteriaceae;g__Acetobacterium;s__Acetobacterium wieringae (bin.29)
- d__Bacteria;p__Bacillota_B;c__Thermincolia;o__Thermincolales;f__UBA2595;g__;s__ (bin.38)
- d__Bacteria;p__Bacillota_C;c__Negativicutes;o__Sporomusales;f__Sporomusaceae;g__;s__ (bin.279)
- d__Bacteria;p__Bacillota_C;c__Negativicutes;o__Sporomusales;f__Sporomusaceae;g__Sporomusa;s__Sporomusa sphaeroides (bin.139)
- d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Tidjanibacter;s__ (bin.81)
- d__Bacteria;p__Elusimicrobiota;c__Endomicrobiia;o__Endomicrobiales;f__Endomicrobiaceae;g__JAHDQW01;s__JAHDQW01 sp018433585 (bin.138)
- d__Bacteria;p__Elusimicrobiota;c__Endomicrobiia;o__Endomicrobiales;f__Endomicrobiaceae;g__JAHDRN01;s__JAHDRN01 sp018433245 (bin.141)
- d__Bacteria;p__Spirochaetota;c__Spirochaetia;o__Sphaerochaetales;f__Sphaerochaetaceae;g__Spiro-02;s__Spiro-02 sp001604275 (bin.151)
- d__Bacteria;p__Synergistota;c__Synergistia;o__Synergistales;f__Synergistaceae;g__Syner-03;s__Syner-03 sp002306075 (bin.235)
- Other

a)

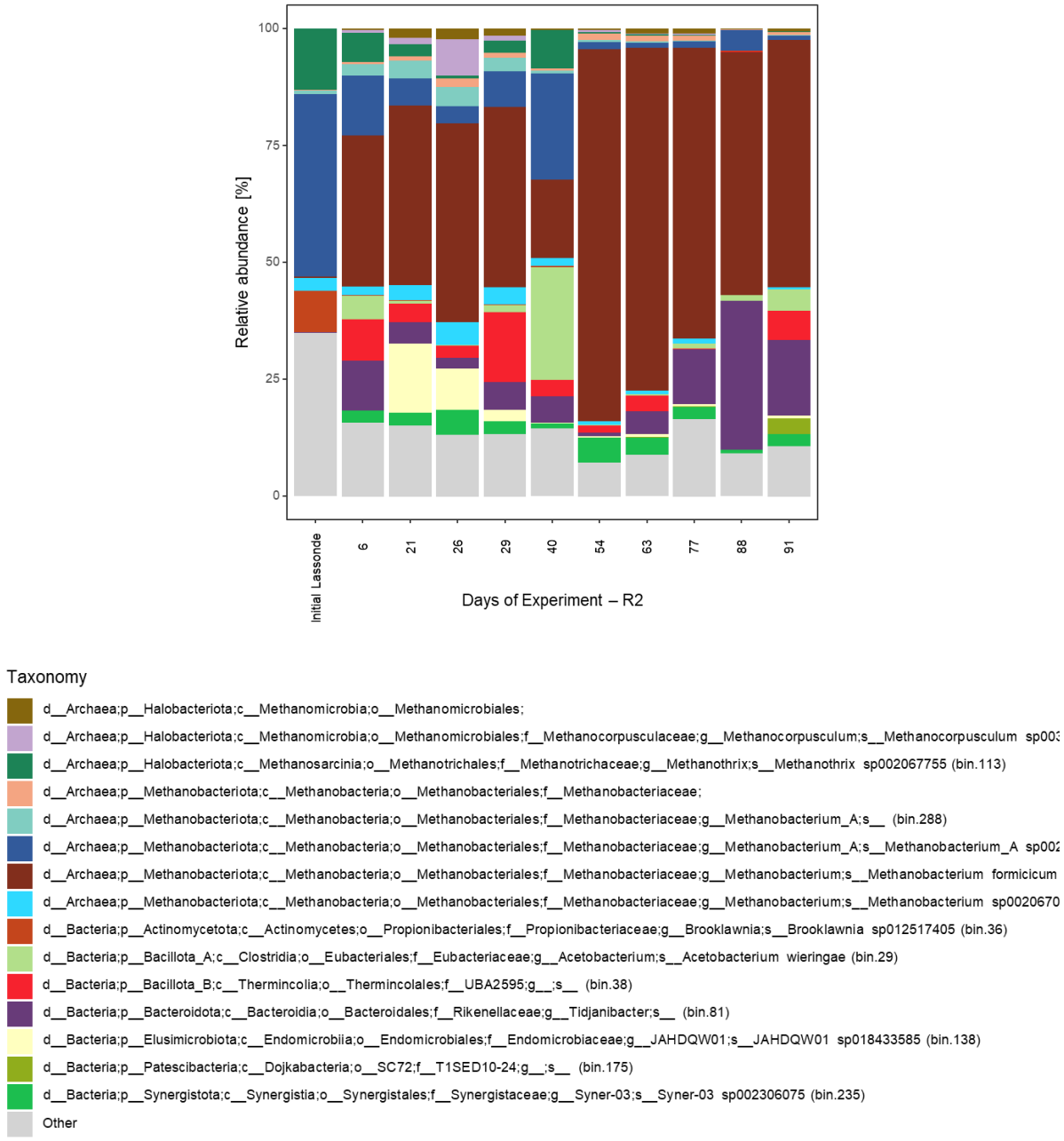


Figure 29) Relative abundance of microbial community of initial sludge compared to liquid in days of experiment of a) R1 and b) R2 in phase 2

Based on the metagenomic analyses of both reactors, there is also a considerable relative abundance of *Tidjanibacter* bacteria in both reactors. Although this genus is less widely discussed in earlier literature, recent studies have started to shed light on its potential roles in anaerobic digestion. *Tidjanibavter* belongs to the *Rikenellaceae* family, a group commonly detected in anaerobic environments. Jiang et al [97] investigating full-scale anaerobic digesters reported that members of the *Rikenellaceae*, including *Tidjanibacter*, are consistently present in the digestate and may be involved in the breakdown of polysaccharides and the production of intermediate volatile fatty acids

(VFAs). These VFAs serve as crucial substrates for methanogens during the subsequent methanogenic phase. In this context, the observed increase in Tidjanibacter abundance in R2 at day 88 likely corresponds to an increased generation of VFAs during experimental sets 12–13. Given that Tidjanibacter species are typically associated with the anaerobic degradation of complex polysaccharides and extracellular polymeric substances (EPS), it is more plausible that the VFAs originated from the hydrolysis and fermentation of these complex biopolymers rather than from direct autotrophic homoacetogenesis from H_2/CO_2. Therefore, the enrichment of Tidjanibacter suggests active breakdown of accumulated extracellular organic matter within the biofilm matrix, contributing to the elevated VFA levels detected during this phase.

Thus, in correlation with the previous results showing that high pressure leads to a more sensitive H_2-consuming population (resulting in lower conversion efficiencies in R2), the consistent relative abundance of *Tidjanibavter* in both reactors suggests that this genus could serve as an important functional buffer in the early digestion steps [98]. By consistently fermenting organic matter into VFAs, Tidjanibacter helps to maintain a steady production of methanogenic substrates (such as acetate), ensuring that the methanogenesis process can continue even when hydrogenotrophic pathways are temporarily inhibited due to excessive dissolved H_2.

4.2.3 Discussion and Implications

In the mentioned investigation, operational constraints limited the maximum pressure in the reactors to 2.5 atm. This limitation may explain the minimal differences observed between R1 and R2, as the pressure range applied might not have been sufficient to elicit significant variations in microbial activity or gas production. Selecting the optimal pressure for anaerobic digestion is closely related to the composition of the feed gas. For instance, in the bioupgrading of CO-rich syngas, higher operating pressures can enhance the solubility of CO, thereby improving its availability to microorganisms and potentially increasing methane production efficiency. However, it is crucial to consider that elevated CO partial pressures can lead to toxicity effects [74]. Conversely, in scenarios involving H_2/CO_2 bioupgrading, maintaining lower pressures is advisable. Elevated pressures can lead to increased hydrogen partial pressures, which may inhibit the activity of hydrogenotrophic methanogens and cause a reduction in pH due to increased CO_2 dissolution, adversely affecting the overall process stability.

Furthermore, although our experiments did not include tests on the other offgases from steel production COG and BOF gas, we can hypothesize based on their typical compositions that differing pressure conditions may be optimal for each. Specifically, COG, which is rich in hydrogen, might perform more efficiently under lower pressure conditions, as reduced pressures can favor hydrogen

reaction kinetics and lower the risks associated with high-pressure hydrogen processing. Conversely, BOF gas, characterized by its higher CO content, could benefit from increased pressures, where the enhanced molecular collision frequency may improve reaction rates and overall conversion efficiency. This conceptual framework highlights the potential for tailoring process conditions to the specific chemical makeup of each offgas stream, suggesting that optimizing pressure settings could be key to maximizing performance in industrial applications.

In conclusion, operating at higher pressures entails increased costs and safety considerations. The need for robust reactor designs to withstand elevated pressures, along with enhanced monitoring and control systems, can significantly escalate operational expenses. Balancing these factors against the potential benefits is crucial. Considering these advantages and disadvantages, a pressure of 1.5 atm was selected for the next phase of our investigation, aiming to optimize performance while mitigating risks and costs.

4.3 Phase 3: Effect of Temperature

In this phase of the study, the focus is on evaluating the effect of temperature on reactor performance by comparing two reactors operating under distinct thermal conditions. R1 functions under mesophilic conditions (36°c), while R2 operates under thermophilic conditions (56°c). Both reactors are maintained at a constant pressure of 1.5 bar and are packed with RFK biocarriers to provide a uniform support matrix for microbial colonization and biofilm development. The feed gas for both reactors is CO 23%, N_2 41%, H_2 15%, CO_2 21 %, reproducing BFG generated when the iron ore is reduced with coke to metallic iron, and table 11 outlines the experimental phases based on variations in the syngas inlet composition. The initial sludge was the mixture of Lassonde and ADM which it has been introduced earlier.

The results of reactor performance, in terms of volumetric efficiency, activity tests, and microbial community analysis, will be presented and discussed in detail in the subsequent sections. This phase aims to highlight the critical role of temperature in shaping reactor performance, microbial activity, and community dynamics, providing valuable insights for optimizing biogas production processes under varying thermal conditions.

Table 11) set of experiments in phase 3

Set	CO (sccm)	N2 (sccm)	H2 (sccm)	CO2 (sccm)	Time(d)
1	10	17.6	6.4	9.2	5.99
2	20	35.2	12.8	18.4	4.75
3	30	52.8	19.2	27.6	3.23
4	40	70.4	25.6	36.8	4.81
5	30	52.8	19.2	27.6	9.03
6	30	100	0	0	10.1
7	25	8.3	0	8.3	6.98
8	30	52.8	19.2	27.6	1.96
9	40	70.4	25.6	36.8	2.07
10	45	79.2	28.8	41.4	3.2

4.3.1 Comparaison: Mesophilic vs. Thermophile Conditions

Figure 30 illustrates the performance of the reactors throught the experimental sets. In the initial phases (1–3) of the experiment, both reactors - R1 (mesophilic) and R2 (thermophilic) - demonstrated comparable performance in terms of COD consumption and methane production, with methane yields exceeding 90%. Notably, R2 achieved an almost complete methane yield of approximately 100%. However, in phase 4, a decline in methane yield was observed in R2, coinciding with increased inlet gas flow rates. Specifically, H_2 consumption efficiency decreased from 100% to 95%, as depicted in figure 30. This reduction suggests that the elevated gas flow rates may have surpassed the gas–liquid mass transfer capacity, leading to insufficient substrate utilization by the microbial community.

Temperature plays a pivotal role in this dynamic. Higher operational temperatures tend to enhance molecular diffusivity, thereby accelerating gas transfer across the interface [99]. Although elevated temperatures also slightly lower the solubility of hydrogen—for instance, its solubility decreases by less than 5% between 35 and 80 °C [100]—this loss is often more than compensated by the improved diffusion kinetics under thermophilic conditions. Nonetheless, if the gas residence time is excessively shortened by very high flow rates. Some studies indicate that higher gas flow rates can result in reduced gas residence time, thereby limiting the availability of substrates like hydrogen for microbial uptake [101]. In contrast, R1 experienced a decrease in both CO and H_2 consumption efficiencies, dropping to 93% and 95%, respectively.

In Phase 5, the experimental conditions were reverted to those of Phase 3 to assess the reactors' recovery capabilities. R2 demonstrated a near-complete restoration in COD consumption and

methane production, with only a minor reduction observed. Specifically, Figure 31 indicates that both CO and H_2 consumption efficiencies in R2 returned to their previous levels, suggesting a full recovery of the CO-consuming microbial population. In contrast, R1 exhibited a 5% decrease in both CO and H_2 consumption compared to Phase 3.

The observed rapid recovery in R2 can be attributed to the accelerated growth rates of thermophilic microorganisms. The thermophilic bacteria are known to have higher metabolic rates, enabling them to adapt swiftly to favorable conditions after a perturbation. This rapid adaptation facilitates efficient substrate utilization and methane production upon returning to optimal conditions. Conversely, mesophilic microorganisms in R1 typically exhibit slower growth rates, which may result in a more gradual recovery following environmental stressors. These findings align with existing literature that highlights the resilience of thermophilic systems [102].

In Phase 6, the experimental introduction of a CO/N_2 mixture aimed to enrich the reactors with CO-consuming microorganisms, followed by feeding under BOF gas composition. R2 demonstrated superior performance, achieving complete CO consumption (100%), whereas R1 exhibited slightly lower CO consumption rates, ranging between 93% and 95%. The enhanced performance of R2 can be attributed to the thermophilic conditions, which favor the activity of specific microbial communities adept at CO utilization. In contrast, mesophilic reactors like R1 often exhibit slower metabolic rates and may harbor microbial communities less specialized in CO degradation, leading to comparatively lower CO consumption efficiencies. The mesophilic conditions may not support the same level of microbial activity and diversity necessary for optimal CO utilization as observed in thermophilic systems.

In Phases 8 and 9, the experimental conditions from Phases 3 and 4 were replicated to assess the reactors' performance under previously tested scenarios. R2 maintained near-complete CO consumption (~100%); however, H_2 consumption declined to 83%. R1 experienced a more pronounced decrease, with both CO and H_2 consumption dropping to 86% in Phase 8 and further to 80% in Phase 9.

As both CO and H_2 consumption decrease below 85% in the mesophilic reactor, the reactor has been stopped while the thermophilic reactor was more pushed to see the maximum capacity of the reactor. Results revealed that althought the methane yield remain more than 95% , a significant decline in H_2 consumption clearly signals a metabolic failure within the thermophilic consortia responsible for syngas biomethanation, even though CO conversion and methane production remain high. The hydrogenotrophic methanogenesis pathway, which relies on the efficient uptake of H_2 to reduce CO_2 to CH_4, appears to be compromised, suggesting that the underlying microbial metabolism is impaired.

This failure is likely due to enzymatic inhibition of key hydrogenases, leading to reduced H_2 uptake [103]. Additionally, the stress imposed by increased feed flow rates under thermophilic conditions may induce shifts in the microbial community that favor CO-metabolizing organisms over hydrogenotrophic methanogens [104]. Such shifts, potentially coupled with substrate overload, create a metabolic bottleneck that critically undermines the utilization of hydrogen, ultimately destabilizing the reactor. This metabolic stress, a well-recognized indicator of system failure, is further supported by Rittmann and McCarty [105], who emphasized that insufficient H_2 consumption compromises the energy balance and stability of anaerobic digestion processes.

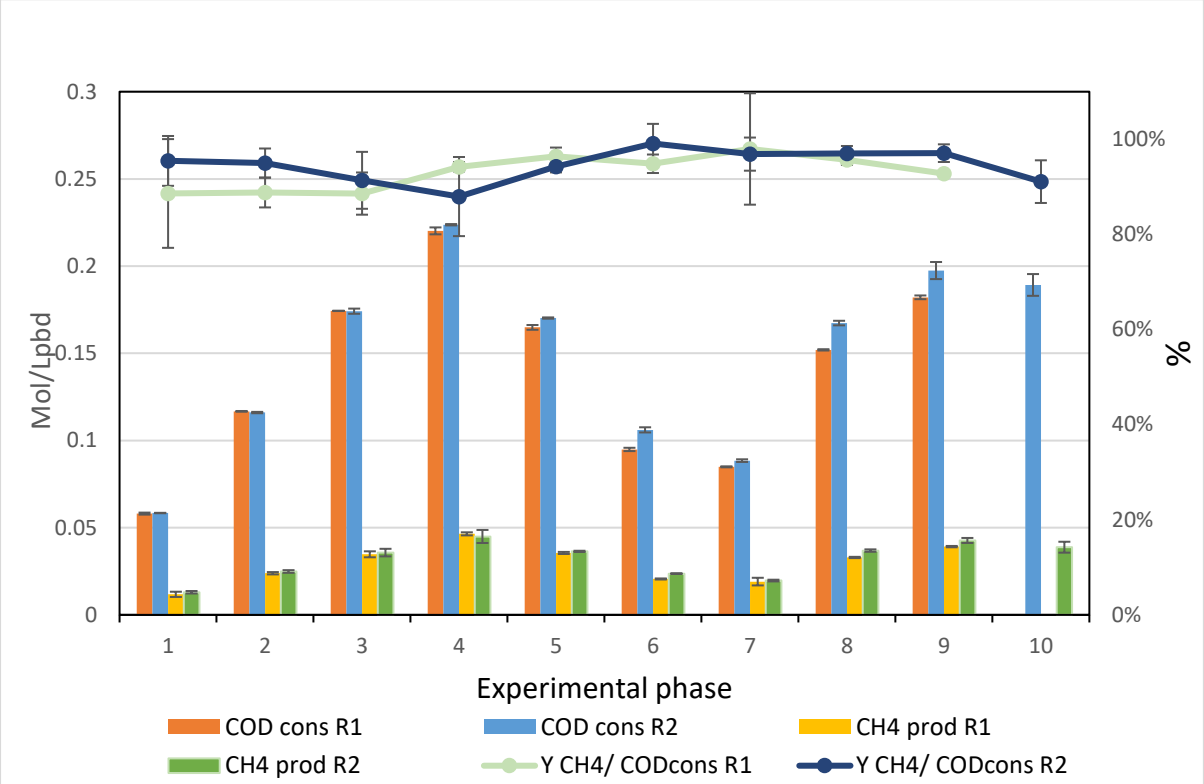


Figure 30) Volumetric methane production, CO consumption and methane production yield of R1 and R2 in phase 3

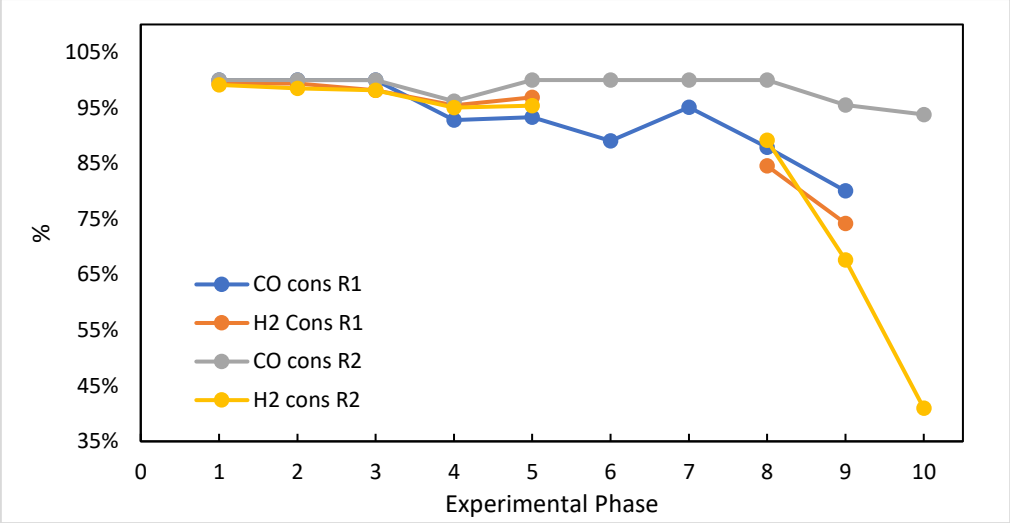


Figure 31) CO and h2 consumption percentage for R1 and R2 in phase 3

The amount of VFA produced and pH of both reactors are presented in figure 32. In R1, VFAs remained zero throughout the process, while in R2, VFA concentrations were elevated, ranging between 200 and 1000 mg/L. Additionally, the pH of R1 was consistently around 0.2 units lower than in R2, fluctuating between 6.2 and 6.5. The absence of VFAs in R1 suggests a balanced microbial ecosystem where acidogenesis and methanogenesis are well-coordinated. In such systems, VFAs produced during the syngas consumption are promptly consumed by methanogens, preventing their accumulation. This efficient syntrophic relationship maintains process stability and ensures optimal methane production.

Conversely, the higher VFA concentrations in R2 indicate an imbalance between acidogenic bacteria and methanogenic archaea. The increase in VFAs observed under thermophilic conditions is attributed to a kinetic imbalance between VFA production and microbial uptake, potentially linked to a shift toward slower syntrophic acetate oxidation pathways. No signs of reactor acidification or community instability were detected. The slightly higher pH in R2 compared to R1 may be attributed to the buffering capacity inherent in thermophilic systems, which can counteract acidification caused by VFA accumulation. However, if VFA production surpasses the system's buffering capacity, it can lead to process instability. Research indicates that maintaining an optimal pH range is essential for maximizing VFA production and ensuring the stability of the microbial community [106].

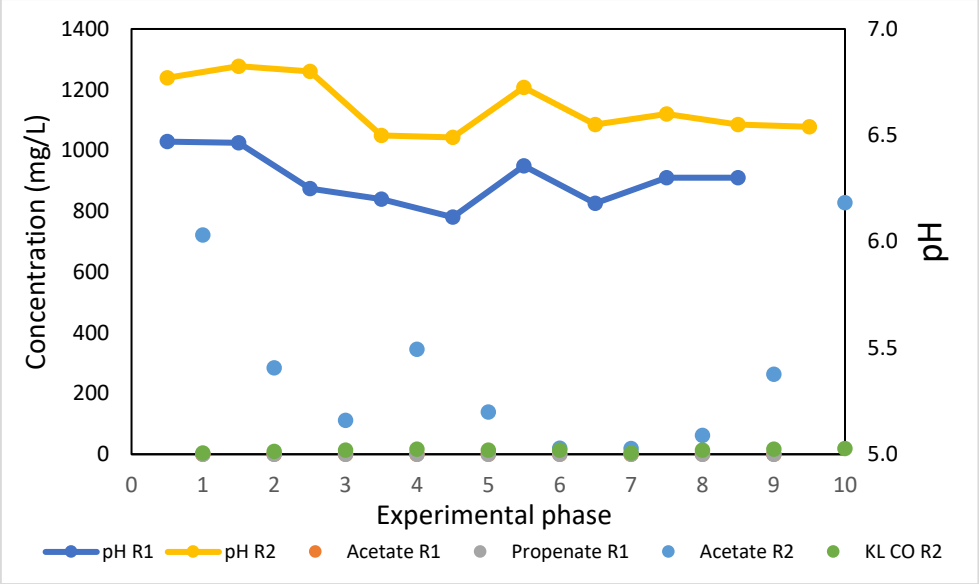


Figure 32) Amount of VFA (namely Acetate and Propionate) and pH for R1 and R2 in phase 3

Figure 33 presented the analysis of VSS per zone across the reactors from initial sludge and after enrichment for both reactors. The contrasting VSS trends between the mesophilic (R1) and thermophilic (R2) reactors can be understood in terms of biofilm structure and microbial yield. There is a hypothesis that in R1, operating under mesophilic conditions, the biomass tends to form a thicker, more voluminous biofilm. This thicker structure is likely a consequence of relatively lower metabolic rates and cell turnover, which permits the accumulation of both cells and extracellular polymeric substances (EPS). The resulting biofilm not only retains more biomass overall—reflected as an increase in VSS—but also allows gradual substrate penetration into its deeper layers, promoting growth even in the inner zones. Conversely, in R2 under thermophilic conditions, the elevated temperature accelerates microbial metabolism, leading to faster substrate uptake and conversion into methane. The higher activity promotes the formation of a more compact, thinner biofilm. With such a streamlined structure, the outer layers of the biofilm rapidly consume the available substrates, leaving the deeper layers relatively starved. This substrate limitation in the inner zones can lead to cell decay or lower biomass yield, which explains the observed reduction in VSS compared to the initial value. Indeed, several studies have noted that thermophilic biofilms are typically thinner and more efficient in mass transfer than their mesophilic counterparts [107]. In other words, while the thermophilic reactor may achieve higher conversion rates and methane productivity due to enhanced kinetics and mass transfer, it does so at the expense of biomass accumulation, resulting in a net reduction in VSS.

Thus, the observed phenomena support the hypothesis that under mesophilic conditions, biofilm growth is more pronounced due to lower substrate consumption rates and higher EPS retention,

whereas the more active thermophilic microbial consortium forms a leaner, more efficient biofilm that, despite its superior activity, accumulates less biomass overall.

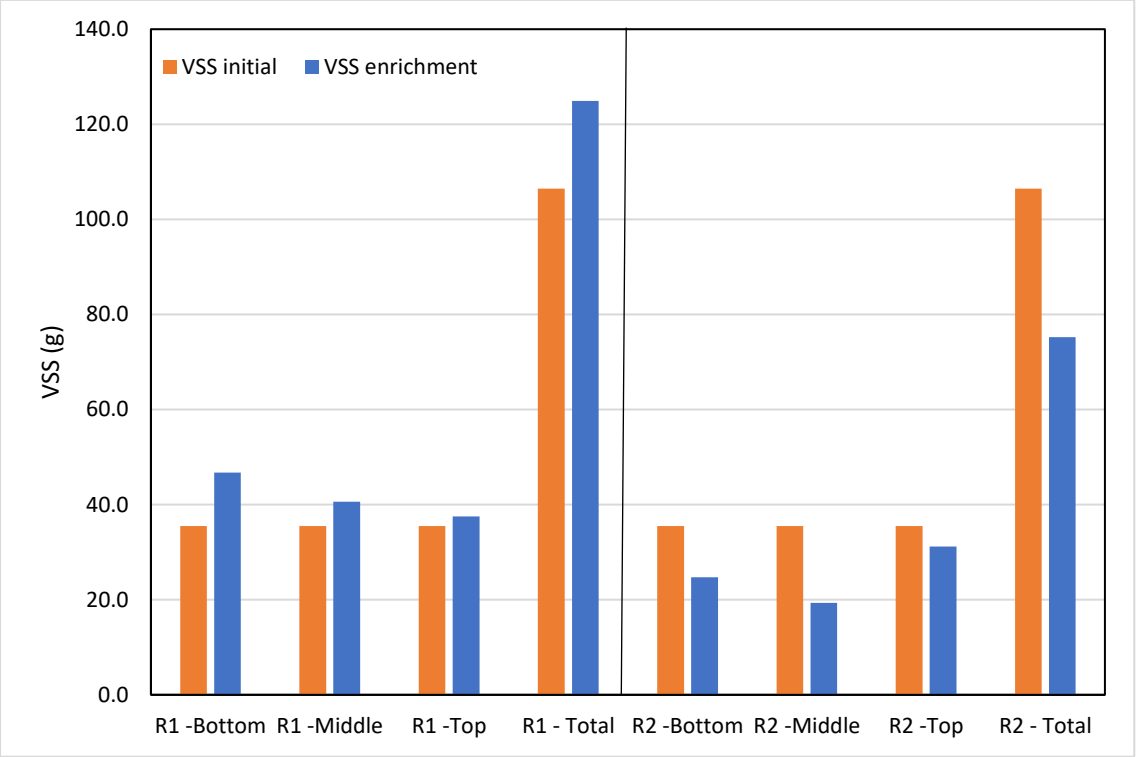


Figure 33) VSS per zone across the reactors from initial sludge and after enrichment for R1 and R2 in phase 3

4.3.3 Temperature and Microbial Activity Correlation

Figure 34 presented the results of different substrate activities and methane production, for initial Lassonde and ADM sludge versus R1 and R2 at the end of experimental phases. Initial CO activities in the inoculum sludges from Lassonde (mesophilic) and ADM (thermophilic) were relatively low, measuring 1.35 and 1.7 mmol/g VSS, respectively. Upon reactor operation, these activities increased substantially, reaching 11.5 mmol/g VSS in R1 and 22.7 mmol/g VSS in R2. This increase first, revealed that TBR are performing well in the enrichment of CO consuming population. Moreover, this significant enhancement in R2 can be attributed to the thermophilic conditions, which are known to accelerate microbial metabolism and enzymatic reactions, leading to higher substrate utilization rates. Thermophilic temperatures can enhance the growth rates of specific microbial populations capable of CO conversion, thereby increasing overall activity [108]. Furthermore, the elevated CO conversion activity in R2 correlates with previous observations of higher methane production and VFA accumulation under thermophilic conditions. The increased microbial activity at higher temperatures can lead to rapid acetogenesis, resulting in VFA accumulation. While VFAs are known to inhibit methanogenic activity when accumulated in high concentrations, the efficient CO conversion observed in R2 suggests that the carboxydotrophic microorganisms remained highly active, converting

CO into intermediate products such as acetate. However, despite this efficient CO conversion, the higher VFA levels detected indicate that the final methanogenic step (conversion of VFAs to methane) may have been partially inhibited. In contrast, R1, operating under mesophilic conditions, exhibited lower CO conversion activity. Mesophilic temperatures are associated with slower microbial growth rates and metabolic activities, which can result in lower substrate utilization rates.

The observed H_2 activity data reveal significant differences between the mesophilic and thermophilic conditions. Initially, the H_2 activity in the mesophilic Lassonde sludge was 70 mmol/g VSS·d, while in the thermophilic ADM sludge, it was substantially higher at 522 mmol/g VSS·d. Upon reactor operation, R1 increased compared to initial Lassonde and decreased compared to ADM in H_2 activity to 334 mmol/g VSS·d, whereas R2 showed a slight increase to 560 mmol/g VSS·d. Notably, the H_2 activity in R2 remained higher than in R1. Thermophilic temperatures are known to optimize the activity of hydrogenase enzymes, which are crucial for hydrogen conversion. Elevated temperatures can increase enzyme activity up to twofold for every 1 °C rise until reaching the optimum temperature, thereby maximizing the efficiency of the hydrogen conversion process. In addition, the elevated temperature improves gas–liquid mass transfer dynamics despite the lower solubility of H_2, ensuring that sufficient substrate reaches the microorganisms. These combined effects may result in a more rapid turnover of H_2 and higher activity in thermophilic systems compared to their mesophilic counterparts after one month of reactor operation.

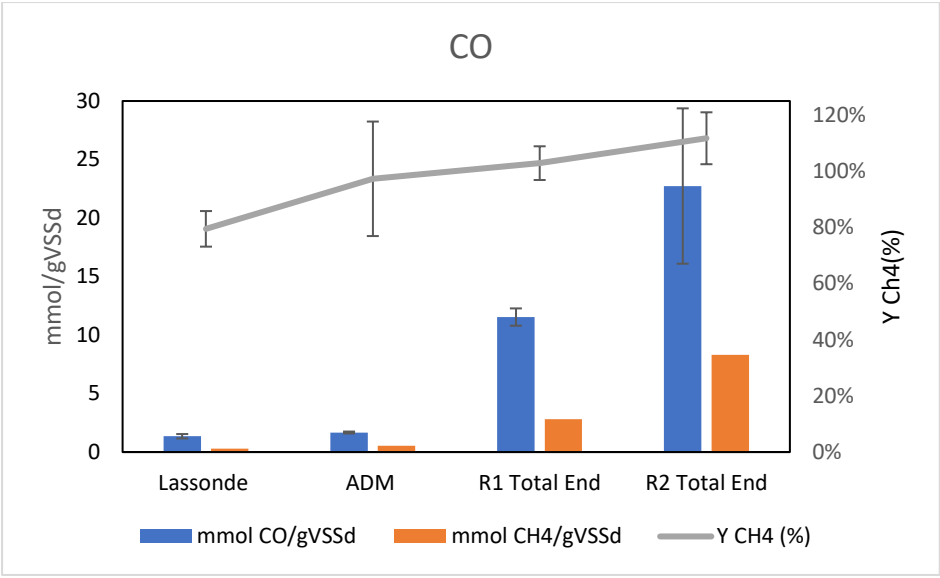
The observed acetate activity data indicate that the R1 maintained an acetate activity of 2.6 mmol/g VSS·d, comparable to the initial Lassonde sludge (2.7 mmol/g VSS·d). In contrast, the thermophilic Reactor R2 exhibited a significant decrease in acetate activity to 0.5 mmol/g VSS·d, despite the ADM sludge's initial higher activity of 10 mmol/g VSS·d.

The microbial community structure in thermophilic reactors tends to differ from that in mesophilic systems. Thermophilic conditions can select specific microbial populations that may have different metabolic capabilities and efficiencies. If the community in R2 is less efficient at acetate consumption or more prone to alternative pathways that do not directly utilize acetate, this could contribute to lowering acetate activity despite high VFA levels. Studies have indicated that thermophilic digestion can lead to distinct microbial consortia with varying capacities for VFA degradation [109].

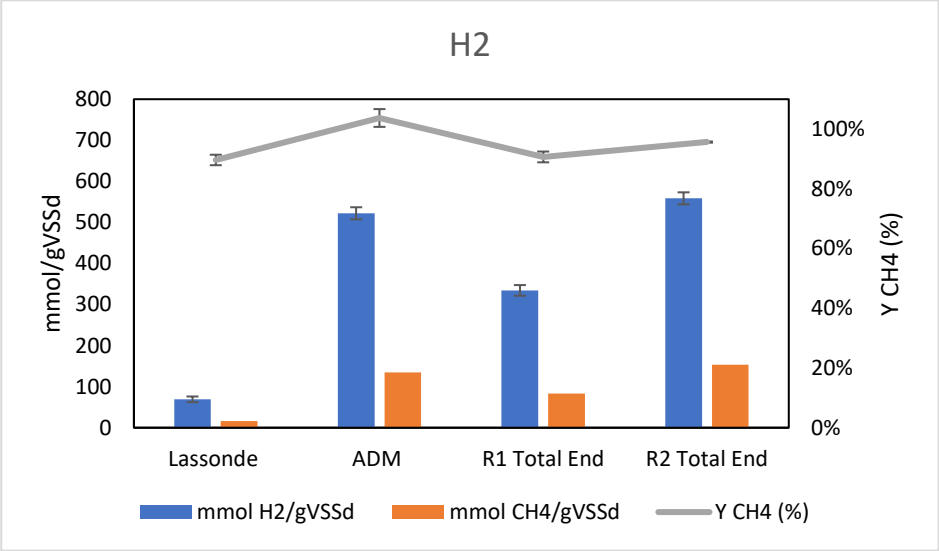
In thermophilic systems, the oxidation of acetate to CO_2 and H_2 by SAO bacteria is thermodynamically challenging under standard conditions. This reaction becomes energetically favorable only when the partial pressure of H_2 is kept very low by rapid consumption from hydrogenotrophic methanogens. If acetate is maintained at a sufficient concentration, it can serve as a substrate for SAO bacteria that “push” electrons toward H_2 production. In other words, with enough acetate available, these

organisms are encouraged to operate the reverse Wood–Ljungdahl pathway in the hydrogenogenic direction rather than running in the reductive (homoacetogenic) mode that consumes H_2 to produce more acetate [110]. Alternatively, some carboxidotrophic bacteria—which oxidize CO—are also known to produce H_2 preferentially when acetate is present. The presence of acetate may signal these organisms to direct electron flow toward H_2 production rather than further acetate formation from H_2 and CO_2. In both cases, a balanced acetate concentration promotes interspecies H_2 transfer: SAO bacteria or carboxidotrophs produce H_2 that is immediately consumed by hydrogenotrophic methanogens, thereby sustaining the overall energy yield and stability of the anaerobic process [111]. In contrast, if acetate levels are too low, homoacetogenic bacteria may dominate by consuming hydrogen and CO_2 to produce acetate, thereby "stealing" electrons from pathways that would otherwise support interspecies hydrogen transfer. This shift could reduce the hydrogen available for hydrogenotrophic methanogenesis [112]. Therefore, it could be important to maintain a certain level of acetate in the thermophilic system

A)



B)



C)

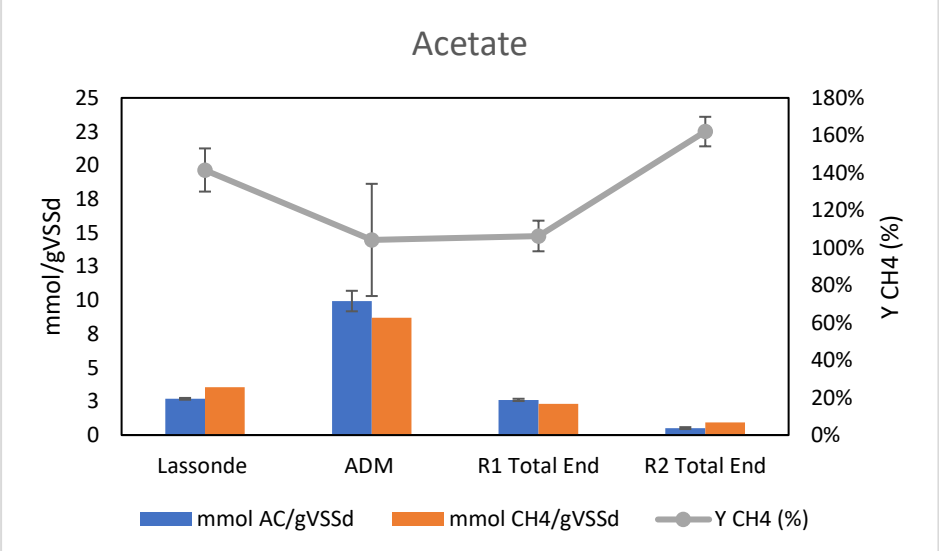


Figure 34) A) CO, B) H_2 and C) Acetate activity, methane production yield results of initial inoculum Lassonde and ADM compared to end of experiments for R1 and R2

4.3.4 Metagenomic Insights of phase 3

In the initial metagenomic analysis for Phase 3 in figure 35, the relative abundance profiles of microbial populations in the initial sludge (Lassonde and ADM) were compared with those in the top, middle, and bottom zones of the biofilm in both R1 and R2. The similarity in the relative abundance of microbial populations across different zones within each reactor indicates a well-mixed system, ensuring uniform microbial distribution. The metagenomic analysis of the biofilm indicates that combining two distinct sludge types maintain and, in some case, promotes microbial diversity.

Thermophilic conditions in R2 promote a broader range of thermophilic microorganisms, potentially enhancing the degradation of substrates and increasing methane production rates. The dominance of specific methanogens in R1 and the diverse community in R2 reflect the influence of operating temperatures on microbial community composition. In R1, the dominance of *Methanobacterium formicicum* (30–40%) is consistent with mesophilic anaerobic digestion environments. *Methanobacterium formicicum* is a hydrogenotrophic methanogen that utilizes hydrogen to reduce CO_2, producing methane. Its prevalence in mesophilic conditions has been documented in studies examining microbial communities in such environments [113].

The biomass in R2 exhibits a more diverse archaeal community, including *Methanoculleus thermophilus* and various *Methanobacteriales* species, as well as the bacterium *Coprothermobacter proteolyticus*. *Methanoculleus thermophilus* is a thermophilic hydrogenotrophic methanogen commonly found in high-temperature anaerobic environments [114]. The presence of *Coprothermobacter proteolyticus*, a thermophilic bacterium known for its proteolytic activity, suggests enhanced protein degradation capabilities in R2. It has been found that these bacteria could enhance biogas production under thermophilic conditions, highlighting their potential in protein-rich waste processing [115]. *C. proteolyticus* degrades complex proteinaceous materials—these may originate from endogenous cell lysis, extracellular polymeric substances (EPS), or co-substrates present in the reactor. This degradation releases amino acids and peptides that can then be fermented by other bacteria. Such fermentation not only recycles nitrogen and carbon within the system but also generates H_2 or VFA that fuel methanogens [116]. Some studies have suggested that certain strains of *C. proteolyticus* might also produce antimicrobial peptides (or microcins), hinting at a possible role in predation or competitive inhibition. Metaproteomic analyses of thermophilic cellulose biomethanisation systems have repeatedly identified abundant proteolytic enzymes from C. proteolyticus, underlining its active role as a “scavenger” of extracellular proteins and a contributor to interspecies hydrogen transfer [117]. This behavior could help shape the microbial community by suppressing less efficient competitors, indirectly favoring a community that is more effective in converting substrates into methane.

The presence of *C. proteolyticus* in R2 is in strong concordance with the VSS data, indicating its significant abundance. Its scavenging activity appears to contribute to an optimal biofilm architecture by maintaining a favorable biofilm thickness.

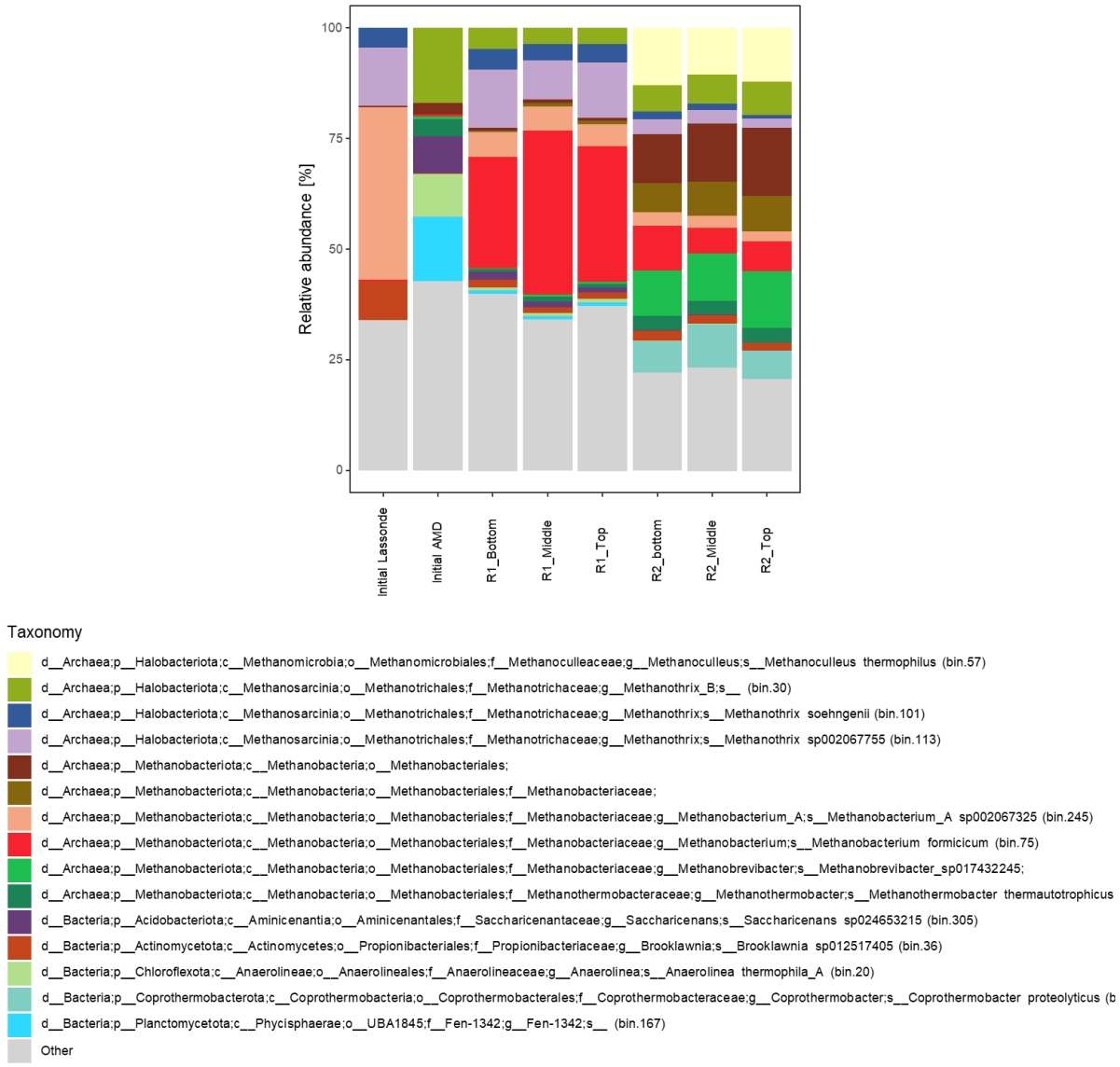
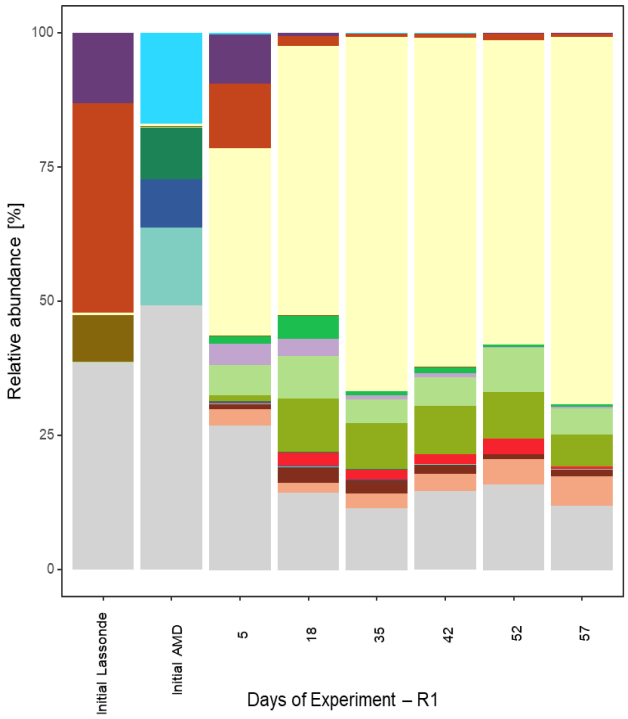


Figure 35) Relative abundance of microbial community of initial sludge (Lassonde and ADM) compared to biofilm in bottom, middle, top zone of R1 and R2 in phase 3

Figure 36 illustrated the relative abundance of microbial communities of liquid media of reactors throughout the days of the experiments. The same trends of biofilm are also in liquid media for both reactors. The high relative abundance of *M. formicicum* in R1 suggests that hydrogenotrophic methanogenesis is by far the predominant pathway in this reactor. The dominance of a single methanogen species may reduce microbial diversity, potentially affecting the system's resilience to environmental fluctuations or changes in substrate composition.

a)



Taxonomy

- d__Archaea;p__Halobacteriota;c__Methanosarcinia;o__Methanotrichales;f__Methanotrichaceae;g__Methanothrix_B;s__ (bin.30)
- d__Archaea;p__Halobacteriota;c__Methanosarcinia;o__Methanotrichales;f__Methanotrichaceae;g__Methanothrix;s__Methanothrix sp002067755 (bin.113)
- d__Archaea;p__Methanobacteriota;c__Methanobacteria;o__Methanobacteriales;f__Methanobacteriaceae;g__Methanobacterium_A;s__Methanobacterium_A sp00
- d__Archaea;p__Methanobacteriota;c__Methanobacteria;o__Methanobacteriales;f__Methanobacteriaceae;g__Methanobacterium;s__Methanobacterium formicicum
- d__Bacteria;p__Actinomycetota;c__Actinomycetes;o__Propionibacteriales;f__Propionibacteriaceae;g__Brooklawnia;s__Brooklawnia sp012517405 (bin.36)
- d__Bacteria;p__Bacillota_A;c__Clostridia;o__Eubacteriales;
- d__Bacteria;p__Bacillota_A;c__Clostridia;o__Eubacteriales;f__Eubacteriaceae;g__Acetobacterium;s__Acetobacterium wieringae (bin.29)
- d__Bacteria;p__Bacillota_B;c__Thermincolia;o__Thermincolales;f__UBA2595;g__;s__ (bin.38)
- d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Tidjanibacter;s__ (bin.81)
- d__Bacteria;p__Chloroflexota;c__Anaerolineae;o__Anaerolineales;f__Anaerolineaceae;g__Anaerolinea;s__Anaerolinea thermophila_A (bin.20)
- d__Bacteria;p__Elusimicrobiota;c__Endomicrobiia;o__Endomicrobiales;f__Endomicrobiaceae;g__JAHDQW01;s__JAHDQW01 sp018433585 (bin.138)
- d__Bacteria;p__Planctomycetota;c__Phycisphaerae;o__Sedimentisphaerales;f__Anaerohalosphaeraceae;g__JAHDQI01;s__ (bin.166)
- d__Bacteria;p__Planctomycetota;c__Phycisphaerae;o__UBA1845;f__Fen-1342;g__Fen-1342;s__ (bin.167)
- d__Bacteria;p__Spirochaetota;c__Spirochaetia;o__Sphaerochaetales;f__Sphaerochaetaceae;g__Spiro-02;s__Spiro-02 sp001604275 (bin.151)
- d__Bacteria;p__Synergistota;c__Synergistia;o__Synergistales;f__Synergistaceae;g__Syner-03;s__Syner-03 sp002306075 (bin.235)
- Other

b)

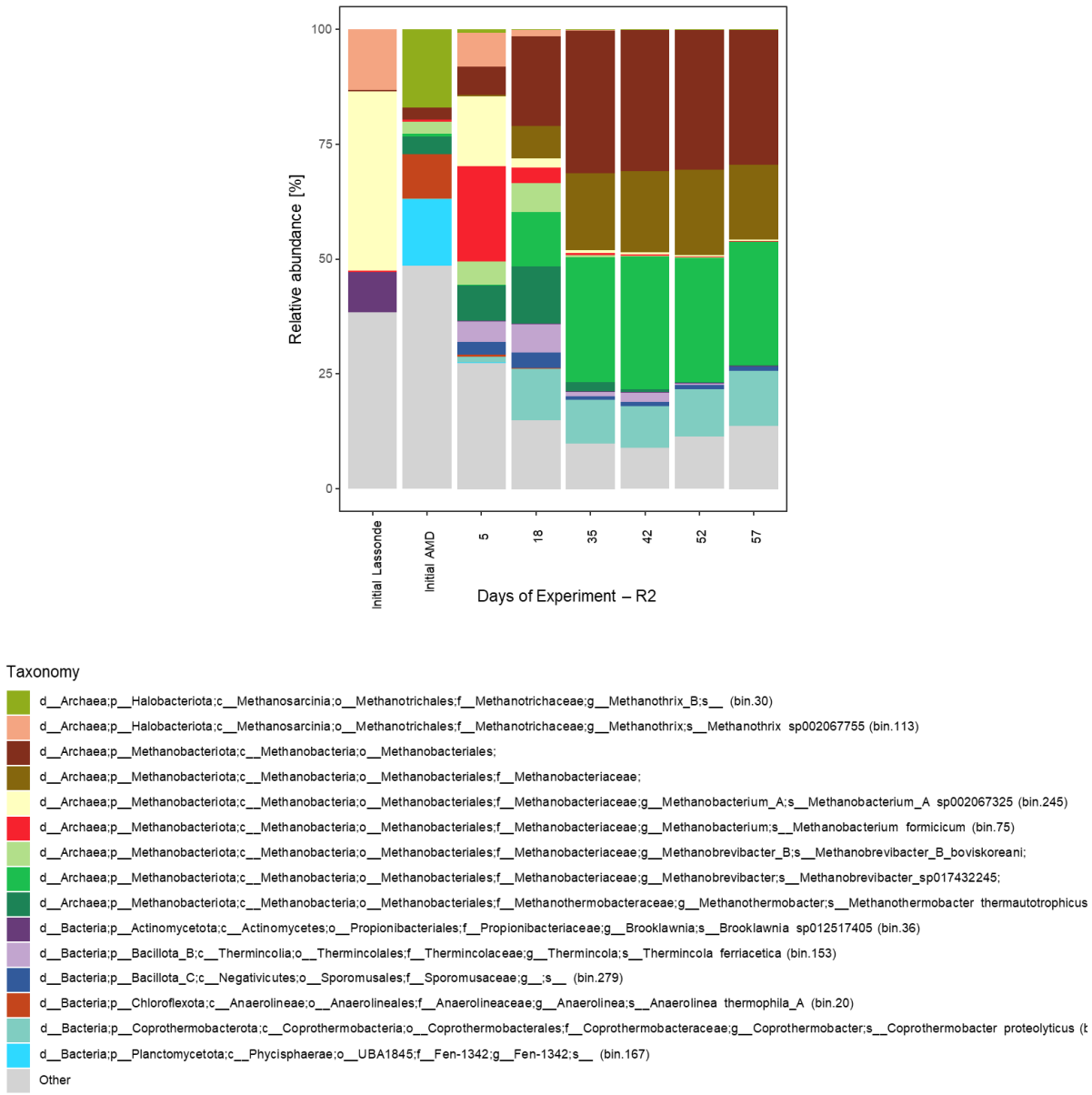


Figure 36) Relative abundance of microbial communities of initial sludge compared to liquid in days of experiment of a) R1 and b) R2 in phase 3

4.3.5 Discussion and Implications

In this phase of our study, R2, operating under thermophilic conditions, demonstrated superior performance in upgrading CO-rich biogas compared to the mesophilic R1. This enhanced performance is evident in several key areas: Thermophilic temperatures accelerate the metabolic rates of microorganisms involved in biomethanation. This increase in metabolic activity leads to more efficient conversion of substrates like CO into methane, which is allied with our result of CO-consumption percentage in R2.

It can be inferred that the mixing of sludges leads to high diversity, resulting in several communities performing the same functions. It's been reported that increased microbial diversity often leads to functional redundancy, where multiple species can perform similar roles, enhancing system stability and resilience. This redundancy suggests that multiple microbial populations can carry out similar metabolic processes, contributing to the overall efficiency and stability of the digestion process [118].

Furthermore, it can be concluded that transitioning from a liquid-based system (e.g. a CSTR) to a biofilm-based configuration (e.g., TBR) appears to reduce the competitive advantage—and hence the abundance—of certain microorganisms that typically thrive under suspended conditions with more complex feedstocks. This reduction likely stems from the spatial and mass transfer constraints inherent to biofilm formation, which can limit the rapid growth of fast-proliferating species. At the same time, the structured nature of biofilms creates a heterogeneous microenvironment that supports a broader spectrum of microbial taxa [113]. Consequently, the overall community diversity in the biofilm is higher than in the recirculating liquid phase, an effect that may enhance process resilience and functional versatility during syngas biomethanation. These observations align with the findings of Ebrahim et al. [59], who reported that, although the biofilm mode may suppress the dominance of certain competitors, it ultimately favors a more diverse and stable microbial consortium conducive to efficient gas conversion.

In general, based on our experimental results, R2, operating under thermophilic conditions, demonstrated superior performance in upgrading CO-rich biogas compared to the mesophilic R1. In addition to these performance metrics, the results from Phase 3 indicate that the thermophilic process in R2 is notably more resilient. The high-temperature environment fosters microbial consortia that not only operate at faster metabolic rates but also exhibit functional redundancy, which is crucial for withstanding fluctuations in substrate composition and operational stresses. This resilience ensures that even under variable conditions, the system can maintain stable performance, thereby enhancing the reliability of CO-rich biogas upgrading in thermophilic digesters.

Note to mention that, the study is subject to several constraints. The microbial consortia used are limited to those readily accessible, which may exclude potentially superior consortia with enhanced performance characteristics. This limits the exploration of diverse microbial community structures and their adaptability under varying conditions. Furthermore, reactor design specifications restrict the operational pressure to a certain threshold, preventing the investigation of higher-pressure scenarios that could yield further insights into methane production optimization. This limitation constrains the ability to explore the full potential of pressure as a critical parameter. Additionally, as the experiments are conducted at the bench scale, scaling up the results for industrial applications might present

challenges, including variations in gas composition, flow dynamics, and long-term microbial stability under continuous operation. These factors must be addressed in future studies to bridge the gap between laboratory findings and industrial implementation. Finally, the study focuses on specific operative parameters and does not explore broader economic or logistical challenges.

4.4 Extra results and observation

4.4.1 Effect of liquid recirculation intervals

In trickle-bed reactors (TBRs) used for the biomethanation of syngas, liquid recirculation plays a crucial role in maintaining effective gas–liquid contact, ensuring that dissolved H_2 and CO_2 are made available to the biofilm-attached methanogens.

Figure 37 indicates that whether the liquid is recirculated intermittently (with a 10% duty cycle, e.g., 1 minute on, 9 minutes off) or continuously for a short period continuously (5-6 minutes per day), the slope of the methane production versus CO removal graph remains nearly identical. This suggests that both recirculation schemes are sufficient to overcome gas–liquid mass transfer limitations under the operating conditions of reactor R1 during phase 1.

This experiment, supported by the literature, is that the key function of recirculation in these systems is to refresh the liquid phase and to maintain a thin, well-distributed film over the packing material. Research has indicated that, beyond a certain threshold, the amount of liquid mixing (and hence the mass transfer rate) is governed more by the reactor's hydrodynamic design and the properties of the biofilm than by the specific recirculation mode used. For example, reviews of biomethanation in TBRs note that if both intermittent and continuous flows provide a comparable total contact time or effective liquid circulation per day, then the overall substrate supply to the microorganisms remains similar, and the system becomes limited by the intrinsic microbial kinetics rather than by mass transfer efficiency [119]. Therefore, it can be concluded that once a minimal effective recirculation regime is established, further variations in the recirculation interval may not significantly alter the overall methane production rate.

Another aspect to consider is that the overall methane production rate in a well-operating TBR is often limited by the metabolic activity of the methanogens. If the organisms are already converting available substrates at their maximum specific rates, then additional improvements in liquid circulation will not further increase methane production. In such cases, as observed in figure 37, both an intermittent regime and a short-duration continuous flow that deliver similar effective recirculation volumes yield nearly identical production kinetics. This observation is consistent with literature findings in biomethanation of syngas, where reactor performance plateaus once the gas–liquid mass transfer has reached a threshold and the process becomes controlled by microbial kinetics [120].

Using a short-duration continuous recirculation - even for only a few minutes per day - was found to promote the formation of a foam layer within the reactor. This foam can be mobilized throughout the reactor, which in turn leads to the displacement of the biofilm from the support material. Since the biofilm is where the hydrogenotrophic methanogens reside and carry out the conversion of CO_2 (or syngas) to CH_4, its displacement is detrimental to reactor performance. By contrast, an intermittent recirculation regime at approximately 10% duty cycle (for example, 1 minute on followed by 9 minutes off) was chosen because it provides sufficient liquid movement to transmit the substrate effectively to the biofilm without generating the turbulent conditions that cause foam formation. In this way, the intermittent flow maintains a stable biofilm while still overcoming the gas–liquid mass transfer limitations that are critical for the biomethanation process.

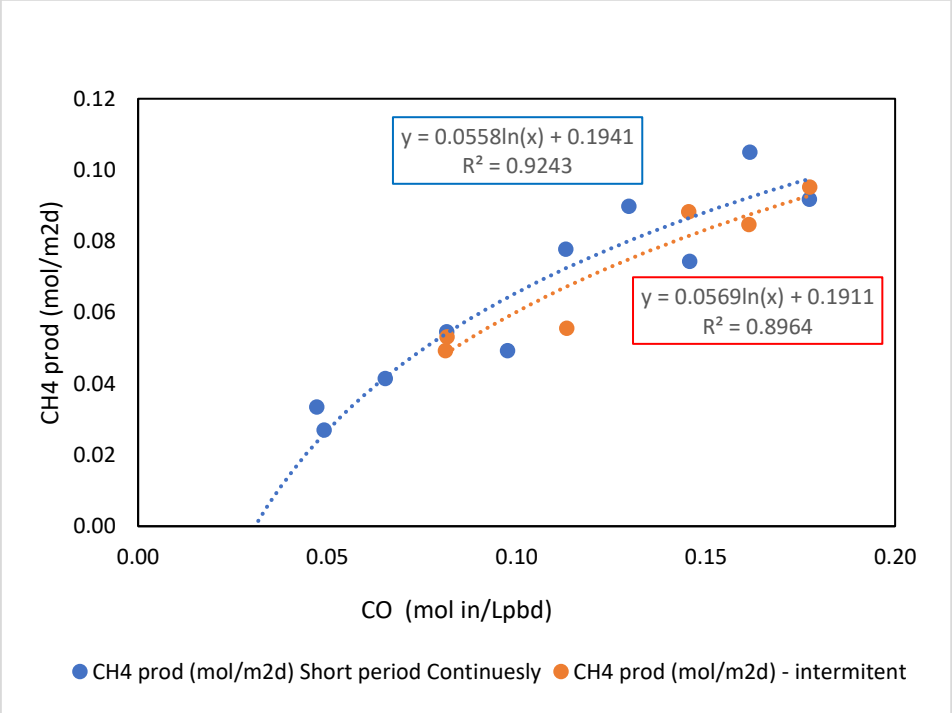


Figure 37) methane production (per surface) based on CO in of reactor1, when the liquid recirculates short period continuously vs intermittent (10% on)

4.4.2 Foam generation phenomena

Foam generation in trickle-bed reactors is typically a multifactorial phenomenon. In the first weeks after inoculation, the microbial community is still adjusting to the reactor environment. Besides possible cell lysis, there may be shifts in community structure where certain species that produce surfactants become temporarily dominant. This shift could further contribute to foaming stabilization and the entrapment of gas bubbles. Under stress conditions (for example, during the acclimation

phase or when substrates are in excess), many microorganisms produce biosurfactants or extracellular polymeric substances (EPS). These surface-active agents reduce surface tension and promote foam stabilization. In some studies, on biomethanation, increases in EPS have been associated with foam generation in the early reactor operation period, even when most cells are still viable [121].

In the early stages following inoculation, much of the observed cell debris can be attributed to a mismatch between the inoculum's microbial composition and the substrate conditions in a syngas biomethanation reactor. The inoculum, sourced from a conventional anaerobic digestion reactor rich in hydrolytic and acidogenic bacteria, is well adapted to environments where organic substrates drive metabolic activity. However, when introduced into a syngas-fed trickle-bed reactor, these trophic groups encounter a starkly different environment—one that lacks the substrates they depend on for survival. As a result, these bacteria quickly experience starvation, leading to cell death and subsequent lysis, which in turn contributes significantly to the accumulation of cell debris. This debris not only reflects the microbial community's adaptation challenges but may also play a role in further reactor phenomena, such as foam stabilization through the entrapment of gas bubbles.

In summary, although dead cell debris from mismatch environment is a valid contributor to foam formation, additional factors such as biosurfactant production, mechanical shear from recirculation, and the inherent physicochemical characteristics of the nutrient medium likely act in concert.

4.4.3 Gas-Liquid Mass Transfer

To determine the volumetric mass transfer coefficient K_{La} for CO in our experimental system, we utilized the relationship between the gas and liquid phases governed by Henry's Law and the observed consumption rate of CO. The mass transfer process was analyzed by considering the equilibrium concentration of CO in the liquid phase and the rate at which CO was consumed by the microbial consortia. The mass transfer rate of CO from the gas phase to the liquid phase can be described by:

$$Q_{CO}\ consumed = K_{La} \times ([C_{CO*}] - [C_{CO}])$$

Rearrange this equation to solve for K_{La} (h^{-1})

$$K_{La} = \frac{Q_{CO}\ consumed}{([C_{CO*}] - [C_{CO}])}$$

Where $Q_{CO}\ consumed$ is the rate of CO consumption, $[C_{CO*}] - [C_{CO}]$ is the driving force for the mass transfer, which is the difference between the equilibrium concentration and the actual concentration.

Henry's Law provides the equilibrium concentration of CO in the liquid phase, which is crucial for understanding the mass transfer dynamics between the gas and liquid phases. It describes the solubility of gases in liquids and is given by the equation:

$$C_{CO*} = H_C \times (P_{total} \times y_{CO})$$

where H_C is Henry's constant for CO, P_{total} is the total pressure in the system, and y_{CO} is the mole fraction of CO in the gas phase. This equation establishes the relationship between the partial pressure of CO in the gas phase and its solubility in the liquid phase. We can rearrange the equation to find K_{La} (h^{-1}):

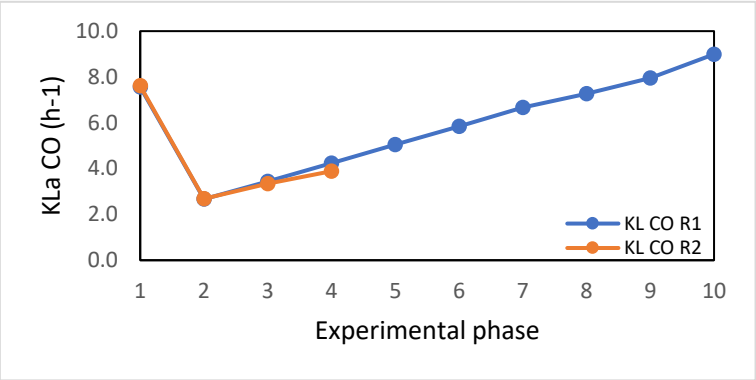
$$K_{La} = \frac{Q_{CO}\ consumed}{([H_C \times (P_{total} \times y_{CO})] - [C_{CO}])}$$

Figure 38 presented the K_{la} CO (h^{-1}) for each experimental sets for all three phases (biocarrier, pressure, temperature). Under the initial set of conditions (Phase 1), both experimental sets yielded identical K_{La} CO values. For biological methanation, the baseline K_{la} is determined predominantly by the interfacial area and the diffusivity of the gas in the liquid phase. In other words, if no additional stress or modification is applied, the system behaves in a predictable manner.

In Phase 2, it is observed that the R1 operated at higher pressure exhibited a consistently lower K_{La} CO. According to Henry's Law, the equilibrium concentration (C_{CO*}) of a gas in a liquid is directly proportional to its partial pressure (P). As Q_{CO} consumed is almost identical in both reactors, increase in pressure thus increases the equilibrium concentration of CO in the liquid phase, thereby enhancing the driving force for the mass transfer which decrease the K_{la} while having the same rate.

In Phase 3, the data showed that, overall, the K_{La} CO values were very similar between the two temperature regimes, with thermophilic conditions sometimes yielding a slightly higher K_{la}. This observation is consistent with literature findings where temperature has a dual effect on gas -liquid mass transfer [122]. With increasing temperature, the solubility of gases in liquids typically decreases, which would tend to reduce the mass transfer driving force. However, higher temperatures lower the liquid's viscosity and increase the molecular diffusivity of the gas, thereby enhancing the K_{La}. These opposing effects may balance each other out so that the net volumetric K_{La} remains relatively unchanged across mesophilic and thermophilic ranges. These insights underscore the importance of considering not only thermodynamic equilibrium (Henry's law) but also hydrodynamic and biological factors when interpreting K_{La} in complex bioreactor systems.

a)



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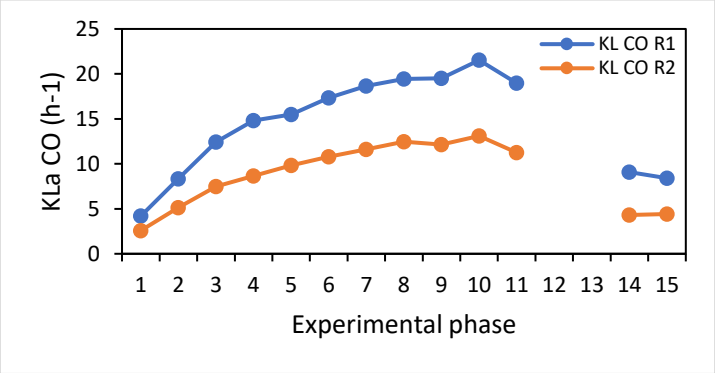

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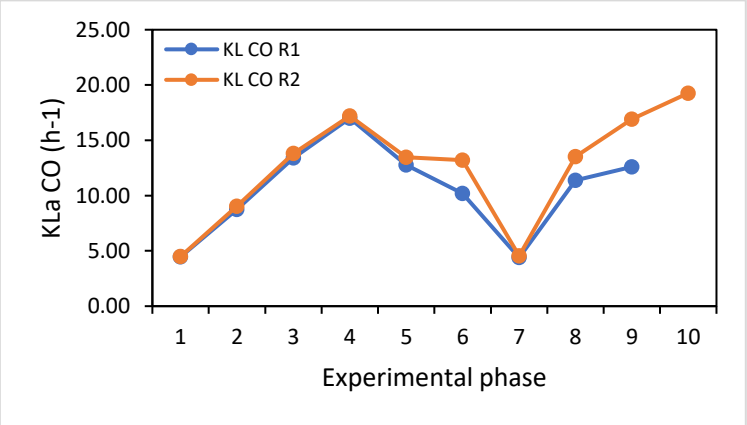

Figure 38) volumetric mass transfer coefficient of CO on a) phase 1, b) phase 2, and c) phase3

5. Conclusions and Recommendations

5.1 Summary of Key Findings

Trickling Bed Reactor configuration offers significant advantages over traditional liquid-based systems like the Continuous Stirred Tank Reactor by effectively enriching and retaining biomass. Activity tests revealed that the TBR supports the formation of robust biofilms on carrier surfaces, thereby increasing local biomass density and minimizing washout issues common in suspended-growth systems. This enhanced biomass retention directly contributes to increased metabolic activity, making the TBR particularly effective for gas-to-methane conversion processes.

Transitioning from a liquid-based to a biofilm-based configuration creates a heterogeneous microenvironment within the reactor. In the TBR, biofilm formation leads to the development of gradients in substrates, pH, and redox conditions. These gradients provide distinct niches that support a broader spectrum of microbial taxa, thereby suppressing the dominance of fast-growing competitors and promoting a diverse and stable microbial consortium. This diversity not only fosters functional redundancy—ensuring continued process performance even when some species are inhibited—but also enhances overall gas conversion efficiency. By promoting functional redundancy, the enhanced microbial diversity ensures that if certain species are inhibited, alternative taxa can take over their metabolic roles, leading to a more robust and efficient conversion of gas substrates, ultimately improving overall gas conversion efficiency.

The efficiency of volumetric methane production was found to be highly dependent on the composition of the inlet gas. Variations in the concentrations of key gases such as H_2 and CO_2, significantly influence the metabolic activities of the methanogenic populations. Changes in gas composition can induce shifts in microbial community structure, impacting both methane yield and reactor stability. For instance, by enriching the inlet gas with additional hydrogen—an approach already proving feasible in industrial biogas upgrading systems—The conversion of CO_2 into methane can be enhanced. This targeted adjustment meets the metabolic needs of dominant methanogens, boosting CO_2 capture and methane yield, while allowing for adaptive management under fluctuating operational conditions.

The generation and retention of biofilm, as measured by VSS (volatile suspended solids), on the biocarriers play a critical role in enhancing the volumetric efficiency of trickle bed reactors (TBRs). Essentially, the greater the surface area provided by the biocarrier; the more biofilm can be accumulated. Even if the per-unit activity of the biomass is relatively lower, the overall increased amount of biomass can compensate by facilitating higher total conversion of substrates into methane. This enhanced biomass accumulation ensures that a larger proportion of the incoming syngas is

effectively utilized, ultimately boosting reactor performance. As a result, the effective surface area of the biocarrier is a key design parameter. A biocarrier with a higher surface area not only supports more extensive biofilm formation but also improves gas–liquid mass transfer, which is crucial for substrate availability to the microorganisms. Increased biofilm mass provides a robust microbial community that can better withstand operational fluctuations and minimize washout, thus maintaining a steady conversion rate. This cumulative effect of enhanced biomass retention and improved mass transfer is central to achieving high volumetric efficiency in TBR systems.

Therefore, when selecting biocarriers for TBR, surface area emerges as a primary criterion. The substantial surface area directly correlates with higher VSS accumulation, which in turn can compensate for any limitations in per-unit biomass activity. Overall, this leads to improve overall performance, making high-surface-area biocarriers the preferred choice for optimizing syngas biomethanation processes in TBRs.

Metagenomic analysis revealed that the microbial community structure was remarkably uniform across all reactor zones, indicating that distinct, spatially separated populations were not established. The relative abundances of key methanogenic and supporting microbial groups were nearly identical from the bottom to the top zones, suggesting that all metabolic pathways and reactions—whether hydrogenotrophic, carboxydotrophic, acetoclastic, or other associated processes—occurred uniformly throughout the reactor. This finding implies that the reactor's design and operational conditions fostered homogeneous mixing and stable environmental conditions, enabling a consistent microbial performance across all zones rather than localized specialized niches.

In phase 2, there the impact of increasing reactor pressure was investigated —up to our operational limit of 2.5 atm—on the biomethanation process using CO-rich syngas. The findings revealed that this pressure increase did not significantly alter the microbial community structure, which remained consistent across the reactor zones, nor did it affect the volumetric methane production efficiency. This suggests that under CO-rich conditions, the reactor system is robust to pressure variations within the tested range. However, it is important to note that for H_2/CO_2-based bioupgrading processes, higher pressures can be problematic. Elevated pressure may lead to a reduction in pH and adversely affect the hydrogen-consuming microbial community, which is more sensitive to such changes. Therefore, while higher pressure appears to be well tolerated in CO-rich syngas conversion, careful consideration is required when applying similar conditions to H_2/CO_2 systems.

In phase 3, where the effect of temperature was investigated, the results revealed distinct advantages of thermophilic over mesophilic conditions. Under thermophilic conditions, the microbial community maintained a broad diversity that enhanced substrate uptake, notably, the enrichment

Coprothermobacter proteolyticus bacteria. The enrichment of *Coprothermobacter proteolyticus* in thermophilic anaerobic systems is highly beneficial, primarily due to its robust scavenging activity. By efficiently degrading cellular debris within the biofilm matrix, this behavior could help shape the microbial community by suppressing less efficient competitors which helps to prevent the over-accumulation of biomass. This controlled biofilm thickness is critical because it enhances the mass transfer, ensuring that nutrients and substrates are more readily available to other metabolically active community members. Moreover, the reduction of detrital buildup appears to foster a more dynamic and active microbial consortium, which can improve overall reactor performance. Consequently, the presence of *C. proteolyticus* not only contributes to nutrient recycling through its proteolytic functions but also indirectly promotes a more efficient and resilient microbial community in thermophilic biogas systems. Overall, although the thermophilic system exhibited a higher degree of VFA accumulation—a potential indicator of the process imbalance—this feature underscores the enhanced capacity of the thermophilic microbial community to degrade substrates. In contrast, the mesophilic conditions in R1 supported a less diverse and more stable microbial community, which, while resulting in more predictable performance, exhibited slower degradation kinetics and lower overall methane production.

In conclusion, the findings demonstrate that thermophilic conditions, despite the challenges associated with VFAs, offer significant advantages in terms of substrate breakdown and methane production, highlighting the importance of optimizing temperature to harness these benefits effectively.

5.2 Implications for the Steel Industry

The steel sector, known for its substantial carbon emissions, is actively seeking methods to mitigate its environmental impact. Trickling Bed Reactors and biofilm-based systems offers promising avenues for transforming waste gases into valuable biofuels. TBRs, through their ability to enrich and retain biomass, create an optimal environment for microbial activity. Activity tests in our study demonstrated that TBR configurations support robust biofilm formation on carrier materials, which increases biomass density, minimizes washout issues, and contributes to efficient gas-to-methane conversion. This biofilm-based system fosters heterogeneous microenvironments, supporting diverse microbial taxa and stabilizing the reactor performance even under fluctuating industrial conditions. Transitioning from traditional liquid-based systems to biofilm-based configurations, creates heterogeneous microenvironments that support diverse microbial communities. This diversity along with the resilience enhances the stability and efficiency of gas conversion processes, which is crucial when dealing with the variable composition of industrial waste gases.

A key advantage of TBRs is the potential to select cost-effective plastic biocarriers that offer a high surface area, promoting extensive biofilm generation. These biocarriers not only reduce the overall capital costs due to their affordability but also enhance microbial attachment and growth, critical for maintaining reactor efficiency. The selection of such materials is crucial in large-scale steel industry applications where economic feasibility and operational efficiency are paramount.

Moreover, maintaining reactor pressure at moderate levels has been shown to sustain high methane production efficiency while simultaneously reducing capital expenditure and mitigating operational safety risks. Lower-pressure systems decrease the need for specialized, high-cost pressure-resistant equipment and reduce the likelihood of pressure-related hazards. Additionally, higher-pressure systems do not appear to significantly enhance volumetric methane production efficiency to an extent that would justify the increased capital (CAPEX) and operational (OPEX) expenditures. While elevated pressure can theoretically improve gas solubility and reaction kinetics, the diminishing returns in the increased cost of pressure-rated reactors, compressors, and safety mechanisms, make moderate-pressure operation a more economically viable choice. Therefore, optimizing reactor pressure within a moderate range strikes a balance between process efficiency, equipment affordability, and operational safety in biomethanation systems. This operational strategy aligns well with the steel industry's stringent safety requirements and cost management goals.

Operating under thermophilic conditions (around 55-60°C) presents another strategic advantage for biogas upgrading within steel plants. The abundant hot gaseous effluents generated during steel production can be harnessed to maintain these elevated temperatures without additional energy input. This synergy not only improves the metabolic rates of thermophilic methanogens, enhancing methane yield, but also leverages waste heat, contributing to the overall energy efficiency of the process.

Furthermore, the composition of inlet gases significantly influences volumetric methane efficiency and microbial community dynamics. Adjustments in the ratios of CO, CO_2, and H_2 can optimize methane production, and metagenomic analyses from our study revealed that such changes also drive shifts in microbial populations, favoring consortia with higher gas conversion capabilities. The steel industry's variable waste gas streams can be effectively managed through adaptive control of these parameters, ensuring consistent biogas output. In this context, supplementing the system with additional H_2—produced via electrolysis—or introducing external sources of CO_2 from neighboring industrial processes can serve as effective strategies to fine-tune these gas ratios. Elevating the H_2 concentration can enhance the activity of hydrogenotrophic methanogens, thereby increasing methane yields. Similarly, augmenting CO_2 levels can stimulate microbial consortia adept at converting CO_2 into

methane, further optimizing biogas production. Therefore, integrating external H_2 and CO_2 sources offers a practical approach to modulate inlet gas compositions, promoting a more efficient and adaptable methane generation process.

5.3 Recommendations for Future Work

Based on the findings of this study, several recommendations can be made to guide future research and optimize the application of TBRs in the steel industry:

Although metagenomic results indicated no significant microbial differences across different reactor zones, future investigations could focus on feeding specific types of inlet gas components at targeted points, such as the middle of the reactor. This approach could provide better control over microbial pathways and reaction kinetics along the reactor length. Exploring zone-based gas feeding strategies may enhance process efficiency and microbial activity.

Given the presence of impurities in the steel industry gaseous effluents, future studies should investigate the effects of these contaminants on biogas production. Introducing artificial effluents, such as sulfur dioxide, into the reactor's inlet gas can help evaluate their inhibitory effects on microbial communities and overall reactor performance. Understanding these impacts will be crucial for designing robust bioreactor systems capable of handling real industrial gas streams.

Scaling up TBRs presents unique challenges compared to CSTRs, particularly in maintaining efficient mass transfer and uniform flow distribution. In TBRs, achieving consistent gas-liquid contact is critical, and scale-up can lead to issues such as channeling, maldistribution, and increased the pressure drop, which adversely affect reactor performance. To address these challenges, it is recommended to conduct comprehensive studies on fluid dynamics within the reactor, possibly utilizing computational fluid dynamics (CFD) modeling, to optimize design parameters such as bed geometry, flow rates, and packing materials. Additionally, pilot-scale experiments should be performed to validate these models and ensure that the scaled-up reactor maintains the desired performance characteristics. Implementing advanced monitoring and control systems can further aid in managing the complexities associated with TBR scale-up, ensuring efficient and safe operation at larger scales.

In syngas biomethanation processes, the maximum conversion rates are often constrained by the metabolic capabilities of the existing microbial consortia. To overcome this limitation and enhance volumetric methane production, it is recommended to explore bioaugmentation strategies. This approach involves introducing specific microbial strains with superior syngas-converting abilities into the reactor's microbial community. By selectively enriching the microbial consortium with such high-

performing strains, it is possible to achieve higher volumetric activity, thereby addressing current bottlenecks in syngas biomethanation processes.

6. References

[1] A. Grimalt-Alemany, I.V. Skiadas, H.N. Gavala, Syngas biomethanation: state-of-the-art review and perspectives, Biofuels, Bioproducts and Biorefining, 12 (2018) 139-158.

[2] J.L. Holechek, H.M. Geli, M.N. Sawalhah, R. Valdez, A global assessment: can renewable energy replace fossil fuels by 2050?, Sustainability, 14 (2022) 4792.

[3] S.D. Angeli, S. Gossler, S. Lichtenberg, G. Kass, A.K. Agrawal, M. Valerius, K.P. Kinzel, O. Deutschmann, Reduction of CO2 emission from off-gases of steel industry by dry reforming of methane, Angewandte Chemie International Edition, 60 (2021) 11852-11857.

[4] A.S. Neto, S. Wainaina, K. Chandolias, P. Piatek, M.J. Taherzadeh, Exploring the Potential of Syngas Fermentation for Recovery of High-Value Resources: A Comprehensive Review, Current Pollution Reports, 11 (2025) 1-18.

[5] M. Mohammadi, G.D. Najafpour, H. Younesi, P. Lahijani, M.H. Uzir, A.R. Mohamed, Bioconversion of synthesis gas to second generation biofuels: A review, Renewable and Sustainable Energy Reviews, 15 (2011) 4255-4273.

[6] I. Angelidaki, L. Treu, P. Tsapekos, G. Luo, S. Campanaro, H. Wenzel, P.G. Kougias, Biogas upgrading and utilization: Current status and perspectives, Biotechnology advances, 36 (2018) 452-466.

[7] K. Svobodova, J.R. Owen, J. Harris, S. Worden, Complexities and contradictions in the global energy transition: A re-evaluation of country-level factors and dependencies, Applied energy, 265 (2020) 114778.

[8] H. Canton, International energy agency—IEA, The Europa Directory of International Organizations 2021, Routledge2021, pp. 684-686.

[9] I. Iron, S. Works, Source: world steel association, 27 February 2020, Wall Street Journal, (2020).

[10] Á.A. Ramírez-Santos, C. Castel, E. Favre, A review of gas separation technologies within emission reduction programs in the iron and steel sector: Current application and development perspectives, Separation and Purification Technology, 194 (2018) 425-442.

[11] A. Talaei, E. Gemechu, A. Kumar, Key factors affecting greenhouse gas emissions in the Canadian industrial sector: A decomposition analysis, Journal of Cleaner Production, 246 (2020) 119026.

[12] W. Uribe-Soto, J.-F. Portha, J.-M. Commenge, L. Falk, A review of thermochemical processes and technologies to use steelworks off-gases, Renewable and Sustainable Energy Reviews, 74 (2017) 809.

[13] A. Frey, V. Goeke, C. Voss, Steel gases as ancient and modern challenging resource; historical review, description of the present, and a daring vision, Chemie Ingenieur Technik, 90 (2018) 1384-1391.

[14] R. Razzaq, C. Li, S. Zhang, Coke oven gas: Availability, properties, purification, and utilization in China, Fuel, 113 (2013) 287-299.

[15] M. Bampaou, K. Panopoulos, P. Seferlis, S. Voutetakis, I. Matino, A. Petrucciani, A. Zaccara, V. Colla, S. Dettori, T. Annunziata Branca, Integration of renewable hydrogen production in steelworks off-gases for the synthesis of methanol and methane, Energies, 14 (2021) 2904.

[16] A. Gaikwad, D. Maga, S. Schlüter, Comparative lifecycle assessment of methanol and urea produced from steel mill gases, Chemie Ingenieur Technik, 94 (2022) 1476-1488.

[17] B. Molitor, H. Richter, M.E. Martin, R.O. Jensen, A. Juminaga, C. Mihalcea, L.T. Angenent, Carbon recovery by fermentation of CO-rich off gases–turning steel mills into biorefineries, Bioresource technology, 215 (2016) 386-396.

[18] A. Steynberg, Introduction to fischer-tropsch technology, Studies in surface science and catalysis, Elsevier2004, pp. 1-63.

[19] J. Lundgren, T. Ekbom, C. Hulteberg, M. Larsson, C.-E. Grip, L. Nilsson, P. Tunå, Methanol production from steel-work off-gases and biomass based synthesis gas, Applied energy, 112 (2013) 431-439.

[20] K.W. Ng, L. Giroux, T. MacPhee, T. Todoschuk, Reduction of blast furnace ironmaking carbon footprint through process integration, AISTech 2010, Proceedings of the iron and steel technology conference, Pittsburgh, PA, USA, (2010), pp. 3-6.

[21] M. Hattori, N. Okuyama, H. Kurosawa, M. Hara, Low-temperature ammonia synthesis on iron catalyst with an electron donor, Journal of the American Chemical Society, 145 (2023) 7888-7897.

[22] N. Schumacher, A. Boisen, S. Dahl, A.A. Gokhale, S. Kandoi, L.C. Grabow, J.A. Dumesic, M. Mavrikakis, I. Chorkendorff, Trends in low-temperature water–gas shift reactivity on transition metals, Journal of Catalysis, 229 (2005) 265-275.

[23] F.M. Liew, M. Köpke, S.D. Simpson, Gas fermentation for commercial biofuels production, Liquid, Gaseous and Solid Biofuels-Conversion Techniques, (2013) 125-173.

[24] N. Canabarro, J.F. Soares, C.G. Anchieta, C.S. Kelling, M.A. Mazutti, Thermochemical processes for biofuels production from biomass, Sustainable Chemical Processes, 1 (2013) 1-10.

[25] S. Dutta, H.N. Gavala, I.V. Skiadas, Scaling up Trickle Bed Reactor for Gas Fermentation Technology: The Effect of Temperature and Reactor Characteristics on Mass Transfer, Fermentation, 10 (2024) 623.

[26] A. Grimalt-Alemany, M. Łężyk, D.M. Kennes-Veiga, I.V. Skiadas, H.N. Gavala, Enrichment of mesophilic and thermophilic mixed microbial consortia for syngas biomethanation: the role of kinetic and thermodynamic competition, Waste and Biomass Valorization, 11 (2020) 465-481.

[27] R. Singh, J. Ryu, S.W. Kim, Microbial consortia including methanotrophs: some benefits of living together, Journal of Microbiology, 57 (2019) 939-952.

[28] Y. Rafrafi, L. Laguillaumie, C. Dumas, Biological methanation of H 2 and CO 2 with mixed cultures: current advances, hurdles and challenges, Waste and Biomass Valorization, 12 (2021) 5259-5282.

[29] E. Oelgeschläger, M. Rother, Carbon monoxide-dependent energy metabolism in anaerobic bacteria and archaea, Archives of microbiology, 190 (2008) 257-269.

[30] J. Sipma, A.M. Henstra, S.N. Parshina, P.N. Lens, G. Lettinga, A.J. Stams, Microbial CO conversions with applications in synthesis gas purification and bio-desulfurization, Critical reviews in biotechnology, 26 (2006) 41-65.

[31] C. Schöne, M. Rother, Methanogenesis from Carbon Monoxide, in: A.J.M. Stams, D.Z. Sousa (Eds.) Biogenesis of Hydrocarbons, Springer International Publishing, Cham, (2019), pp. 123-151.

[32] G. Diekert, G. Wohlfarth, Metabolism of homoacetogens, Antonie Van Leeuwenhoek, 66 (1994) 209-221.

[33] S. Hattori, Syntrophic acetate-oxidizing microbes in methanogenic environments, Microbes and environments, 23 (2008) 118-127.

[34] J.R. Phillips, R.L. Huhnke, H.K. Atiyeh, Syngas fermentation: a microbial conversion process of gaseous substrates to various products, Fermentation, 3 (2017) 28.

[35] R. Kleerebezem, M.C. van Loosdrecht, Mixed culture biotechnology for bioenergy production, Current opinion in biotechnology, 18 (2007) 207-212.

[36] R. Muñoz, L. Meier, I. Diaz, D. Jeison, A review on the state-of-the-art of physical/chemical and biological technologies for biogas upgrading, Reviews in Environmental Science and Bio/Technology, 14 (2015) 727-759.

[37] B. Lóránt, G.M. Tardy, Current status of biological biogas upgrading technologies, Periodica Polytechnica Chemical Engineering, 66 (2022) 465-481.

[38] S. Rahat, Biological biomethanation: in-situ, ex-situ and hybrid methods for upgrading of biogas or syngas, (2023) 10-11.

[39] J. Figueras, H. Benbelkacem, C. Dumas, P. Buffiere, Syngas biomethanation: In a transfer limited process, is CO inhibition an issue?, Waste Management, 162 (2023) 36-42.

[40] A. Grimalt-Alemany, M. Łężyk, L. Lange, I.V. Skiadas, H.N. Gavala, Enrichment of syngas-converting mixed microbial consortia for ethanol production and thermodynamics-based design of enrichment strategies, Biotechnology for biofuels, 11 (2018) 1-22.

[41] H. Jiang, F. Wu, Y. Wang, L. Feng, H. Zhou, Y. Li, Characteristics of in-situ hydrogen biomethanation at mesophilic and thermophilic temperatures, Bioresource technology, 337 (2021) 125455.

[42] N. Lawson, M. Alvarado-Morales, P. Tsapekos, I. Angelidaki, Techno-economic assessment of biological biogas upgrading based on danish biogas plants, Energies, 14 (2021) 8252.

[43] P.C. Munasinghe, S.K. Khanal, Syngas fermentation to biofuel: evaluation of carbon monoxide mass transfer coefficient (kLa) in different reactor configurations, Biotechnology progress, 26 (2010) 1616-1621.

[44] F.S. Mederos, J. Ancheyta, J. Chen, Review on criteria to ensure ideal behaviors in trickle-bed reactors, Applied Catalysis A: General, 355 (2009) 1-19.

[45] J.J. Orgill, H.K. Atiyeh, M. Devarapalli, J.R. Phillips, R.S. Lewis, R.L. Huhnke, A comparison of mass transfer coefficients between trickle-bed, hollow fiber membrane and stirred tank reactors, Bioresource technology, 133 (2013) 340-346.

[46] V. Alopaeus, K. Hynynen, J. Aittamaa, A cellular automata model for liquid distribution in trickle bed reactors, Chemical engineering science, 61 (2006) 4930-4943.

[47] T.L. Dupnock, M.A. Deshusses, High-performance biogas upgrading using a biotrickling filter and hydrogenotrophic methanogens, Applied biochemistry and biotechnology, 183 (2017) 488-502.

[48] M. Koutinas, L.G. Peeva, A.G. Livingston, An attempt to compare the performance of bioscrubbers and biotrickling filters for degradation of ethyl acetate in gas streams, Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology, 80 (2005) 1252-1260.

[49] K. Klasson, J. Cowger, C. Ko, J. Vega, E. Clausen, J. Gaddy, Methane production from synthesis gas using a mixed culture of R. rubrum M. barkeri, and M. formicicum, Applied biochemistry and biotechnology, 24 (1990) 317-328.

[50] K. Asimakopoulos, H.N. Gavala, I.V. Skiadas, Biomethanation of syngas by enriched mixed anaerobic consortia in trickle bed reactors, Waste and Biomass Valorization, 11 (2020) 495-512.

[51] M. Burkhardt, T. Koschack, G. Busch, Biocatalytic methanation of hydrogen and carbon dioxide in an anaerobic three-phase system, Bioresource technology, 178 (2015) 330-333.

[52] A. Chatzis, E. Orellana, M. Gaspari, K. Kontogiannopoulos, L. Treu, A. Zouboulis, P.G. Kougias, Comparative study on packing materials for improved biological methanation in trickle Bed reactors, Bioresource Technology, 385 (2023) 129456.

[53] F. Morgan-Sagastume, Biofilm development, activity and the modification of carrier material surface properties in moving-bed biofilm reactors (MBBRs) for wastewater treatment, Critical reviews in environmental science and technology, 48 (2018) 439-470.

[54] M.B. Jensen, L.D.M. Ottosen, M.V.W. Kofoed, H2 gas-liquid mass transfer: A key element in biological Power-to-Gas methanation, Renewable and Sustainable Energy Reviews, 147 (2021).

[55] Q. Zune, S. Telek, S. Calvo, T. Salmon, M. Alchihab, D. Toye, F. Delvigne, Influence of liquid phase hydrodynamics on biofilm formation on structured packing: Optimization of surfactin production from Bacillus amyloliquefaciens, Chemical Engineering Science, 170 (2017) 628-638.

[56] P.G. Lutran, K.M. Ng, E.P. Delikat, Liquid distribution in trickle-beds. An experimental study using computer-assisted tomography, Industrial & Engineering Chemistry Research, 30 (1991) 1270-1280.

[57] I. Stamatiou, F.L. Muller, Determination of mass transfer resistances in trickle bed reactors, Chemical Engineering Journal, 377 (2019) 119808.

[58] Z. Kusnere, K. Spalvins, D. Blumberga, I. Veidenbergs, Packing materials for biotrickling filters used in biogas upgrading–biomethanation, (2021) 10-11.

[59] F. Ebrahimian, N. De Bernardini, P. Tsapekos, L. Treu, X. Zhu, S. Campanaro, K. Karimi, I. Angelidaki, Effect of pressure on biomethanation process and spatial stratification of microbial communities in trickle bed reactors under decreasing gas retention time, Bioresource technology, 361 (2022) 127701.

[60] E.M. Goonesekera, A. Grimalt-Alemany, E. Thanasoula, H.F. Yousif, M.C. Valerin, I. Angelidaki, Mass Transfer in the Biofilm Layer and Thermodynamic Constraints Shape Microbiome Ecology in Syngas Biomethanation Trickle Bed Reactors, (2024) 4901390.

[61] K. Asimakopoulos, M. Łężyk, A. Grimalt-Alemany, A. Melas, Z. Wen, H.N. Gavala, I.V. Skiadas, Temperature effects on syngas biomethanation performed in a trickle bed reactor, Chemical Engineering Journal, 393 (2020) 124739.

[62] Z. Yang, Y. Liu, J. Zhang, K. Mao, M. Kurbonova, G. Liu, R. Zhang, W. Wang, Improvement of biofuel recovery from food waste by integration of anaerobic digestion, digestate pyrolysis and syngas biomethanation under mesophilic and thermophilic conditions, Journal of Cleaner Production, 256 (2020) 120594.

[63] S. Hupfauf, A. Winkler, A.O. Wagner, S.M. Podmirseg, H. Insam, Biomethanation at 45 C offers high process efficiency and supports hygienisation, Bioresource technology, 300 (2020) 122671.

[64] S.R. Guiot, R. Cimpoia, G. Carayon, Potential of wastewater-treating anaerobic granules for biomethanation of synthesis gas, Environmental science & technology, 45 (2011) 2006-2012.

[65] R. Conrad, B. Wetter, Influence of temperature on energetics of hydrogen metabolism in homoacetogenic, methanogenic, and other anaerobic bacteria, Archives of Microbiology, 155 (1990) 94-98.

[66] T. Ullrich, J. Lindner, K. Bär, F. Mörs, F. Graf, A. Lemmer, Influence of operating pressure on the biological hydrogen methanation in trickle-bed reactors, Bioresource technology, 247 (2018) 7-13.

[67] K.T. Klasson, M.D. Ackerson, E.C. Clausen, J.L. Gaddy, Bioconversion of synthesis gas into liquid or gaseous fuels, (1992).

[68] W. Merkle, K. Baer, J. Lindner, S. Zielonka, F. Ortloff, F. Graf, T. Kolb, T. Jungbluth, A. Lemmer, Influence of pressures up to 50 bar on two-stage anaerobic digestion, Bioresource technology, 232 (2017) 72-78.

[69] L.-M. Mauerhofer, S. Zwirtmayr, P. Pappenreiter, S. Bernacchi, A.H. Seifert, B. Reischl, T. Schmider, R.-S. Taubner, C. Paulik, S.K.-M. Rittmann, Hyperthermophilic methanogenic archaea act as high-pressure CH4 cell factories, Communications Biology, 4 (2021) 289.

[70] K.M. Hurst, R.S. Lewis, Carbon monoxide partial pressure effects on the metabolic process of syngas fermentation, Biochemical Engineering Journal, 48 (2010) 159-165.

[71] J.M. O'Brien, R. Wolkin, T. Moench, J. Morgan, J. Zeikus, Association of hydrogen metabolism with unitrophic or mixotrophic growth of Methanosarcina barkeri on carbon monoxide, Journal of bacteriology, 158 (1984) 373-375.

[72] D.E. LaRowe, A.W. Dale, J.P. Amend, P. Van Cappellen, Thermodynamic limitations on microbially catalyzed reaction rates, Geochimica et Cosmochimica Acta, 90 (2012) 96-109.

[73] H. Horn, E. Morgenroth, Transport of oxygen, sodium chloride, and sodium nitrate in biofilms, Chemical Engineering Science, 61 (2006) 1347-1356.

[74] S. Sancho Navarro, R. Cimpoia, G. Bruant, S.R. Guiot, Biomethanation of syngas using anaerobic sludge: shift in the catabolic routes with the CO partial pressure increase, Frontiers in microbiology, 7 (2016) 1188.

[75] J. Sipma, P.N. Lens, A.J. Stams, G. Lettinga, Carbon monoxide conversion by anaerobic bioreactor sludges, FEMS Microbiology Ecology, 44 (2003) 271-277.

[76] D. Wise, C. Cooney, D. Augenstein, Biomethanation: Anaerobic fermentation of CO2, H2 and CO to methane, Biotechnology and bioengineering, 20 (1978) 1153-1172.

[77] A. Kapic, S.T. Jones, T.J. Heindel, Carbon monoxide mass transfer in a syngas mixture, Industrial & Engineering Chemistry Research, 45 (2006) 9150-9155.

[78] M.D. Bredwell, P. Srivastava, R.M. Worden, Reactor design issues for synthesis-gas fermentations, Biotechnology progress, 15 (1999) 834-844.

[79] S. Hashemi, S.E. Hashemi, K.M. Lien, J.J. Lamb, Molecular microbial community analysis as an analysis tool for optimal biogas production, Microorganisms, 9 (2021) 1162.

[80] B. Niya, K. Yaakoubi, F.Z. Beraich, M. Arouch, I.M. Kadmiri, Current status and future developments of assessing microbiome composition and dynamics in anaerobic digestion systems using metagenomic approaches, Heliyon, (2024).

[81] L. Xiao, E. Lichtfouse, P.S. Kumar, Advantage of conductive materials on interspecies electron transfer-independent acetoclastic methanogenesis: A critical review, Fuel, 305 (2021) 121577.

[82] J. Tremblay, L. Schreiber, C.W. Greer, High-resolution shotgun metagenomics: the more data, the better?, Briefings in Bioinformatics, 23 (2022) bbac443.

[83] C.-D. Dubé, S.R. Guiot, Ethanol-to-methane activity of Geobacter-deprived anaerobic granules enhanced by conductive microparticles, Process Biochemistry, 63 (2017) 42-48.

[84] C.-Y. Lee, C.-H. Lin, Bacterial growth and substrate degradation by BTX-oxidizing culture in response to salt stress, Journal of Industrial Microbiology and Biotechnology, 33 (2006) 37-44.

[85] W. Boyles, Chemical oxygen demand, Technical information series, Booklet,(9), 24 (1997).

[86] B. Tartakovsky, F. Lebrun, S.R. Guiot, C. Bock, A comparison of microbial and bioelectrochemical approaches for biogas upgrade through carbon dioxide conversion to methane, Sustainable Energy Technologies and Assessments, 45 (2021) 101158.

[87] Y. Zhao, M. Haddad, R. Cimpoia, Z. Liu, S.R. Guiot, Performance of a Carboxydothermus hydrogenoformans-immobilizing membrane reactor for syngas upgrading into hydrogen, International journal of hydrogen energy, 38 (2013) 2167-2175.

[88] Y. Chen, J.J. Cheng, K.S. Creamer, Inhibition of anaerobic digestion process: a review, Bioresource technology, 99 (2008) 4044-4064.

[89] Y. Liu, W.B. Whitman, Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea, Annals of the new York Academy of Sciences, 1125 (2008) 171-189.

[90] B.A. Berghuis, F.B. Yu, F. Schulz, P.C. Blainey, T. Woyke, S.R. Quake, Hydrogenotrophic methanogenesis in archaeal phylum Verstraetearchaeota reveals the shared ancestry of all methanogens, Proceedings of the National Academy of Sciences, 116 (2019) 5037-5044.

[91] S. Langer, D. Schropp, F.R. Bengelsdorf, M. Othman, M. Kazda, Dynamics of biofilm formation during anaerobic digestion of organic waste, Anaerobe, 29 (2014) 44-51.

[92] K.A. Taconi, M.E. Zappi, W.T. French, L.R. Brown, Methanogenesis under acidic pH conditions in a semi-continuous reactor system, Bioresource Technology, 99 (2008) 8075-8081.

[93] D. Poggio, A. Sastraatmaja, M. Walker, S. Michailos, W. Nimmo, M. Pourkashanian, Experimental evaluation of continuous in-situ biomethanation of CO2 in anaerobic digesters fed on sewage sludge and food waste and the influence of hydrogen gas–liquid mass transfer, Processes, 11 (2023) 604.

[94] L. Chen, S. Du, L. Xie, Effects of pH on ex-situ biomethanation with hydrogenotrophic methanogens under thermophilic and extreme-thermophilic conditions, Journal of bioscience and bioengineering, 131 (2021) 168-175.

[95] R.K. Thauer, A.-K. Kaster, H. Seedorf, W. Buckel, R. Hedderich, Methanogenic archaea: ecologically relevant differences in energy conservation, Nature Reviews Microbiology, 6 (2008) 579-591.

[96] G. Luo, I. Angelidaki, Integrated biogas upgrading and hydrogen utilization in an anaerobic reactor containing enriched hydrogenotrophic methanogenic culture, Biotechnology and bioengineering, 109 (2012) 2729-2736.

[97] C. Jiang, M. Peces, M.H. Andersen, S. Kucheryavskiy, M. Nierychlo, E. Yashiro, K.S. Andersen, R.H. Kirkegaard, L. Hao, J. Høgh, Characterizing the growing microorganisms at species level in 46 anaerobic digesters at Danish wastewater treatment plants: A six-year survey on microbial community structure and key drivers, Water research, 193 (2021) 116871.

[98] L. Zhang, X. Gong, L. Wang, K. Guo, S. Cao, Y. Zhou, Metagenomic insights into the effect of thermal hydrolysis pre-treatment on microbial community of an anaerobic digestion system, Science of The Total Environment, 791 (2021) 148096.

[99] A. Pauss, G. Andre, M. Perrier, S.R. Guiot, Liquid-to-gas mass transfer in anaerobic processes: inevitable transfer limitations of methane and hydrogen in the biomethanation process, Applied and environmental microbiology, 56 (1990) 1636-1644.

[100] J. Sonne-Hansen, P. Westermann, B.K. Ahring, Kinetics of sulfate and hydrogen uptake by the thermophilic sulfate-reducing bacteria Thermodesulfobacterium sp. strain JSP and Thermodesulfovibrio sp. strain R1Ha3, Applied and environmental microbiology, 65 (1999) 1304-1307.

[101] B. Jiang, D. Zhang, X. Hu, U. Söderlind, G. Paladino, S. Gamage, E. Hedenström, W. Zhang, J. Arrigoni, A. Lundgren, Low-Grade Syngas Biomethanation in Continuous Reactors with Respect to Gas–Liquid Mass Transfer and Reactor Start-Up Strategy, Fermentation, 9 (2022) 38.

[102] M. Kim, Y.-H. Ahn, R. Speece, Comparative process stability and efficiency of anaerobic digestion; mesophilic vs. thermophilic, Water research, 36 (2002) 4369-4385.

[103] L. Braga Nan, E. Trably, G. Santa-Catalina, N. Bernet, J.-P. Delgenès, R. Escudié, Biomethanation processes: new insights on the effect of a high H 2 partial pressure on microbial communities, Biotechnology for biofuels, 13 (2020) 1-17.

[104] G.A.W. Sudiartha, T. Imai, C. Mamimin, A. Reungsang, Effects of temperature shifts on microbial communities and biogas production: an in-depth comparison, Fermentation, 9 (2023) 642.

[105] B. Rittmann, Environmental Biotechnology: Principles and Applications, McGraw-Hill, (2001).

[106] M. Atasoy, Z. Cetecioglu, The effects of pH on the production of volatile fatty acids and microbial dynamics in long-term reactor operation, Journal of Environmental Management, 319 (2022) 115700.

[107] D. Lu, H. Bai, B. Liao, Comparison between Thermophilic and Mesophilic Membrane-Aerated Biofilm Reactors—A Modeling Study, Membranes, 12 (2022) 418.

[108] M. Diender, P.S. Uhl, J.H. Bitter, A.J. Stams, D.Z. Sousa, High rate biomethanation of carbon monoxide-rich gases via a thermophilic synthetic coculture, ACS sustainable chemistry & engineering, 6 (2018) 2169-2176.

[109] A.O. Wagner, E.M. Prem, R. Markt, R. Kaufmann, P. Illmer, Formation of phenylacetic acid and phenylpropionic acid under different overload conditions during mesophilic and thermophilic anaerobic digestion, Biotechnology for biofuels, 12 (2019) 1-24.

[110] C. Li, L. Hao, F. Lü, H. Duan, H. Zhang, P. He, Syntrophic acetate-oxidizing microbial consortia enriched from full-scale mesophilic food waste anaerobic digesters showing high biodiversity and functional redundancy, Msystems, 7 (2022) e00339-00322.

[111] A.M. Henstra, A.J. Stams, Deep conversion of carbon monoxide to hydrogen and formation of acetate by the anaerobic thermophile Carboxydothermus hydrogenoformans, International journal of microbiology, 2011 (2011) 641582.

[112] N.M.C. Saady, Homoacetogenesis during hydrogen production by mixed cultures dark fermentation: unresolved challenge, International Journal of Hydrogen Energy, 38 (2013) 13172-13191.

[113] G. Cheng, F. Gabler, L. Pizzul, H. Olsson, Å. Nordberg, A. Schnürer, Microbial community development during syngas methanation in a trickle bed reactor with various nutrient sources, Applied Microbiology and Biotechnology, 106 (2022) 5317-5333.

[114] J.-L. Garcia, B. Ollivier, W.B. Whitman, The order methanomicrobiales, Prokaryotes, 3 (2006) 208-230.

[115] K. Tandishabo, K. Nakamura, K. Umetsu, K. Takamizawa, Distribution and role of Coprothermobacter spp. in anaerobic digesters, Journal of bioscience and bioengineering, 114 (2012) 518-520.

[116] P. Liczbiński, S. Borowski, A. Nowak, Isolation and Use of Coprothermobacter spp. to Improve Anaerobic Thermophilic Digestion of Grass, Molecules, 27 (2022) 4338.

[117] F. Lü, A. Bize, A. Guillot, V. Monnet, C. Madigou, O. Chapleur, L. Mazéas, P. He, T. Bouchez, Metaproteomics of cellulose methanisation under thermophilic conditions reveals a surprisingly high proteolytic activity, The ISME journal, 8 (2014) 88-102.

[118] F. Puig-Castellví, L. Cardona, D. Jouan-Rimbaud Bouveresse, C.B. Cordella, L. Mazéas, D.N. Rutledge, O. Chapleur, Assessment of the microbial interplay during anaerobic co-digestion of wastewater sludge using common components analysis, PLoS One, 15 (2020) e0232324.

[119] C.F. Fenske, D. Strübing, K. Koch, Biological methanation in trickle bed reactors-a critical review, Bioresource Technology, 385 (2023) 129383.

[120] N. Aryal, M. Odde, C.B. Petersen, L.D.M. Ottosen, M.V.W. Kofoed, Methane production from syngas using a trickle-bed reactor setup, Bioresource Technology, 333 (2021) 125183.

[121] P. Kougias, K. Boe, S. O-Thong, L. Kristensen, I. Angelidaki, Anaerobic digestion foaming in full-scale biogas plants: a survey on causes and solutions, Water science and technology, 69 (2014) 889.

[122] A. Ferreira, C. Ferreira, J. Teixeira, F. Rocha, Temperature and solid properties effects on gas–liquid mass transfer, Chemical Engineering Journal, 162 (2010) 743-752.