COST-EFFECTIVE GRANULAR SLUDGE-BASED PROCESS FOR DISTILLERY WASTEWATER TREATMENT AND REUSE

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1. EXECUTIVE SUMMARY

The distilleries industry is among the most water-intensive and polluting industries in the world. The resulting distillery wastewater has an extremely high concentration of organic contaminants, nutrients and solids. Development of cost-effective, energy-efficient and reliable distillery wastewater (pre-) treatment processes before releasing to the municipal sewer system is urgently needed, in order to avoid the over-strength surcharge, reduce stress to City's WWTP and meet Distiller's GHG reduction and water reuse goals. Currently, there is no single distillery wastewater treatment process that is cost-effective and energy-efficient, due to the complex nature of contaminants in the distillery wastewater.

This study aimed to optimize and demonstrate a cost-effective and resource recovery-based distillery wastewater treatment processes at the pilot-scale. In collaboration with Alberta Distillers, Dr. Liu and the research team developed and optimized integrated pilot-scale two-stage granular sludge (GS)-based bioreactors for distillery wastewater treatment, focusing on energy recovery, nutrients reduction, and water reuse, and achieved over 98% biological oxygen demand (BOD) removal and 99% ammonia removal within a short hydraulic retention time.

To help better understand the linkage between reactor performance and microbial community dynamics, the biochemical and molecular tools developed in Dr. Liu's laboratories were used to advance the knowledge of the physicochemical and biological properties and activities of the microorganisms in granular sludge under various operation conditions. Control strategies were developed to optimize energy, nutrient and water recovery. These activities provided valuable information for process development, and guided future full-scale, cost-effective implementation of the granular sludge-based reactor systems for distillery wastewater treatment and reuse.

2. INTRODUCTION

2.1 Sector Introduction

2.1.1 Challenges with distillery wastewater treatment

The distillery industries represent a key contributor to the global economy; however, they are also among the most polluting industries in the world. Distillery wastewater (DWW) generated during alcohol

production is characterized by its large volume and high strength. On average, 8-15 L of DWW is produced for every litre of ethanol produced [1]. The total volume of DWW generated from the distillery industries in Canada is estimated to be ~ 0.74 million m^3/day [2-4], which accounts for ~4.5% of the nation's total municipal wastewater discharged daily [5].

The DWW, either generated from the distillery process (i.e., process wastewater containing primarily distillery stillage, which is the bottom discharge from distillation, also called spent wash) [6] or from cooling, fermenter cleaning, or bottling processes (i.e., non-process wastewater), are highly polluted wastewaters [6-8]. In general, process DWW is acidic, dark brown in colour, and with high chemical oxygen demand (COD, > 80,000 mg/L), total suspended solids (TSS, > 2,400 mg/L), total nitrogen (TN, ~ 5,000 mg/L), and total phosphorous (TP, ~ 2,500 mg/L) [8]. In comparison, water quality of non-process DWW varies significantly depending on the source, with fermenter cleaning DWW (FC-DWW) being the most concentrated and difficult to treat, with high COD (>20,000 mg/L), high salinity (total dissolved solids (TDS) >80,000 mg/L), extremely high pH (>13.5), high TN (~400 mg /L), and TP (> 157 mg/L). Other non-process DWW streams have significantly lower organics and nutrient concentrations. Currently, no treatment process is available for either process or non-process DWW treatment.

2.2 Technology Gaps

Despite the large volume and treatment needs, cost-effective and energy-efficient DWW treatment is currently not available [3, 6, 8, 10-14]. In fact, for most distilleries, the current practice is to mix all process and non-process DWW streams together, adjust pH and then dilute to meet sewer discharge guideline, before its discharge to the sewer collection systems for treatment at the centralized wastewater treatment plants (WWTP). This approach (i) is extremely water and chemical wasting, (ii) increases operation costs, and (iii) adds loading to the centralized WWTPs. Although some engineering firms offer DWW treatment designs for distillers, the designs are not optimized; and in most cases, all DWW streams are still mixed, and pH adjusted before on-site treatment. Further, this approach misses the opportunity for biomethane and nutrient recovery from high-strength wastewater, water reuse from low-strength wastewater, and has extremely high capital and operational costs. High-strength process wastewater treatment has been heavily focused on energy-intensive physiochemical processes [15-20], and low performance has been widely reported for biological treatment due to the lack of system optimization and process control [21-24]. Cost-effective DWW treatment technologies are in urgent needs.

3. PROJECT DESCRIPTION

3.1 Technology Description

3.1.1 Technology innovation

Over the past year, Dr. Liu's team has completed a feasibility study for DWW management. Characterization of wastewater generated from different sources (both process and non-process wastewaters) was conducted. Assessment of different mixing options has been conducted. A new approach has been developed to holistically manage DWW.

3.1.1A Maximizing resource recovery and reducing chemical addition for distillery wastewater treatment: considering wastewater characteristics from different waste streams

In addition to mixing DWW generated from different sources, it is more important to identify highly polluted DWW for energy and nutrient recovery and lightly polluted DWW for water reuse within the distillers. In particular, caustic wastewater (non-process wastewater) and centrate wastewater (process wastewater) should be managed for COD, N, P reduction, and pH adjustment. All other wastewater streams are lightly polluted and can be easily treated using biological/physiochemical processes depending on the reuse options. A better approach is to mix spent caustic and centrate waste streams, which has been assessed and approved successful in previous study. This is particularly important because the pH adjustment is expensive, and is a safety concern due to the need in handling strong acid/base for the neutralization purpose.

3.1.1B Optimizing treatment efficiency through new reactor design, operation and selection of optimal microbial communities

Design and operation considerations such as reactor configuration, mixing regime, feeding strategies, temperature, recirculation, and hydraulic retention time (HRT) have significant impacts on bioreactor microbial community development and active microbial biomass retention, which should be optimized to improve reactor performance [25-28]. As such, selection of high-rate and robust reactor designs is critical to achieve desired treatment. Here, a two-stage granular sludge (GS)-based process was employed, including a high-rate anaerobic GS (An-GS) process (Stage I) for organics removal, biomethane and phosphorous recovery, and an aerobic/anoxic GS (A/O-GS) process (Stage II) for energy-efficient residual COD and ammonia reduction. Both processes were recently developed in Dr. Liu's labs and are being operated at lab- and pilot-scales for different types of high-strength wastewater.

Stage I: Anaerobic granular sludge reactor (An-GS). The proposed An-GS process is based on a recently patented process by Dr. Liu's team: Simultaneous Phosphorus Recovery in Energy Generation reactor (in short: SPRING) [29]. The design is based on the modified operation of upflow anaerobic sludge blanket (UASB) reactors and can simultaneously recover biomethane and phosphorus (through phosphorous precipitation in granular sludge) from wastewater at high methane production and organic loading rates. Phosphorus precipitation further supports granule formation, and the sludge has been shown to be highly resistant to environmental and operation stress and shock-loading conditions. Thus the An-GS is suitable for organics reduction from high-strength wastewater [29, 30], and has been tested for DWW treatment at the laboratory-scale.

Stage II: Aerobic/anoxic granular sludge reactor (A/O-GS). During anaerobic digestion (AD), organic nitrogen in proteins, amino acids, and uric acids is hydrolyzed to inorganic ammonia [31], therefore an increase of ammonia concentration in the AD reactor effluent is often observed. Hence, a polishing step following An-GS is needed given the high concentrations of organics and nutrients in DWW, in order to meet discharge limits. The proposed A/O-GS process is highly cost-effective for high-ammonia wastewater treatment and was also recently developed in Dr. Liu's group [32]. In particular, the A/O granules are dense microbial consortia packed with different microbial species and contain millions of organisms per

gram of biomass that have the necessary physiological capabilities to degrade wastewater pollutants. The A/O granule structure supports the establishment of nitritation/denitritation/anammox in a single granule, which helps reduce the sludge production and reactor footprint. Thus, the A/O-GS reactor is a good option to be placed after An-GS as a post-treatment.

3.1.1C Integrated An-GS and A/O-GS processes for distillery wastewater treatment

Over the past year, Dr. Liu's team has conducted treatability studies of high-strength DWW generated at the AB Distillers at the laboratory-scale. The two-stage An-A/O-GS system was effective for DWW treatment and resource recovery. In particular, An-GS can be operated at relatively high organic loading conditions (>10 kg COD/m^3/d) for effective biomethane and phosphorous recovery, and A/O-GS is effective for post COD and ammonia reduction with relatively low HRT required (< 10 hours). Optimization of the two-stage reactor operation and process control is needed to further improve treatment efficiency, while minimizing energy consumption and reactor footprint. This is a critical step for future implementation of the two-stage GS process for DWW management.

3.1.1D Key features of the integrated An-GS and A/O-GS processes

The integrated An-A/O-GS technology is a two-stage high-strength granular sludge (GS)-based wastewater treatment technology, with a small footprint, low energy requirements, and low maintenance requirements (e.g., sludge disposal only required once or twice a year).

High-rate reactor system for high-strength wastewater treatment. With the advanced engineering design, the An-A/O-GS system cultivates small-sized granular sludge using improved bioreactor design on hydraulics and microbiome shear strength that favors densely packed and fast settling sludge, with 5 times more increase in density and sludge settleability, compared with conventional anaerobic continuous stirred tank reactors and activated sludge system. Particularly, the An-GS processes is specially designed to simultaneously recover energy and phosphorous from wastewater containing high COD and nutrients. The granule structure of the anaerobic sludge provided a protective layer to sensitive methanogens; thus, the An-GS systems can perform robustly under shock loading and toxic conditions. High methane production (up to 2.5 NL CH_4/L/d), high organic loading rates (~15 kg COD/m^3/d), and high phosphorous recovery (> 80% PO_4-P) in the form of $Ca_3(PO_4)_2$ precipitates were achieved with the An-GS process for high-strength wastewater (e.g. DWW, food-processing wastewater, brown water and blackwater) [29]. The A/O-GS process is tailored to provide a high-activity microbial community targeting ammonia removal. It is a new generation cost-effective technology with smart control and energy-efficient engineering design that has been optimized to achieve 100% of ammonia removal from high-ammonia wastewater (e.g. landfill leachate wastewater, and anaerobic/aerobic digester sludge liquor) at a very short treatment retention time consistently [32].

High energy efficiency. The An-GS process is based on the energy-efficient AD process, while the A/O-GS process incorporates an all-in-one energy-efficient biological ammonia reduction pathway, where nitritation-denitritation-anammox all occur in a single granule. Different from the conventional nitrification/denitrification processes, the A/O-GS process significantly reduces aeration energy requirements and does not need chemical addition for ammonia treatment.

Simple operation and low maintenance requirements. The integrated process provides a cost-effective solution for high-strength wastewater treatment (particularly for removing COD, ammonia and phosphorous), with extremely low sludge production (sludge disposal is only required once or twice a year) and a very small footprint (around one-tenth of the size of regular reactors). The treated effluent also meets the City's sewer systems discharge requirements without the overstrength burden.

Modular design. The modular design of the An-GS and A/O-GS reactors make it easy to add or reduce the number of units needed by increasing or reducing the treatment capacity as well as for the higher or lower DWW strength. It is common for DWW to have variations in the contaminate concentrations based on the raw materials, chemicals, and processes used by the distillers. This flexibility in treatment arrangement and layout provided a significant advantage for the integrated process over other treatment processes and technologies on the market as there are no known DWW treatment processes with a footprint as small and performance as high as the proposed integrated process. Hence, it is expected that the integrated process to be developed can be directly implemented at the Alberta Distillers and other distillers in Alberta, Canada and globally.

3.1.2 Objectives

This study aimed to develop and optimize a cost-effective process for the treatment of high-strength DWW at the pilot-scale. To overcome the potential inhibitions from the high-strength DWW, the granular sludge (GS)-based process was selected because of its high tolerance towards shock loading conditions and effectiveness in contaminant removal [33, 34].

Specifically, this project developed and optimized a two-stage granular sludge (GS)-based process (An-GS followed by A/O-GS) to treat DWW at the pilot-scale at the Alberta Distillers (Figure 1). The team focused on improving process energy efficiency and bioenergy and phosphorous recovery, as well as reducing chemical costs needed for pH adjustment, and exploring opportunities to enrich active anaerobic microbial communities for biomethane and P recovery in the Stage I reactor, and to develop energy efficient nitrogen reduction pathway in the Stage II reactor. Information regarding process control, process footprint and waste management strategies was collected for future full-scale process development.

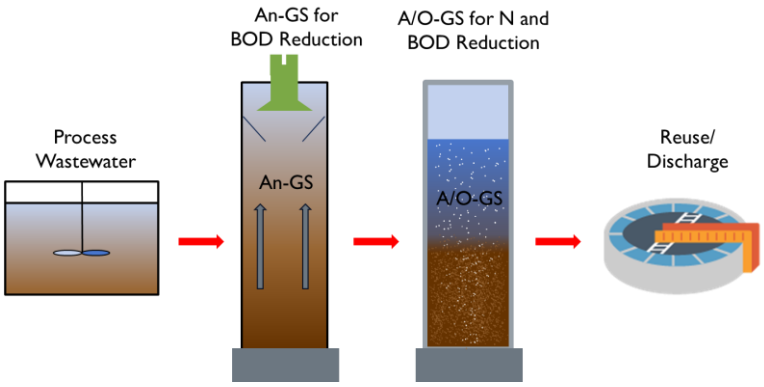

Figure 1. Project image

3.2 Updates to Project Objectives

There were no changes to the original objectives.

3.3 Performance Metrics

3.3.1 Clean Resources Metrics

Metric	Project Target	Current Status	Comments
TRL advancement	7	7	Pilot-scale GSR operation successfully completed.
Field pilots/demonstrations	1	1	The proposed process demonstrated at AB Distillers at the pilot-scale.
Students Trained (M.Sc., Ph.D., Postdoc)	4	10	Ten HQPs have been trained, including • 3 MSc students • 4 PhD students • 3 Postdocs
Publications	2	3	One published, two submitted.
Knowledge Mobilization	2	5	Four project meetings and one conference presentation.
New products/services created	1	1	A new process for distillery wastewater treatment verified. Design for full-scale demonstration has been proposed to the partner.

3.3.2 Program Specific Metrics

Metric	Project Target	Current Status
% Improvement of overall water use efficiency	Treated effluent meets or exceeds discharge standards.	Exceeds

3.3.3 Project Success Metrics

Metric	Project Target	Current status	Comments
Training of HQPs	4 HQPs successfully trained in this project	10 HQPs trained.	Ten HQPs have been trained, including • 3 MSc students • 4 PhD students • 3 Postdocs

Design of integrated process for full-scale application	A final design will be formulated to integrate both GS-based processes	Achieved	Design for full-scale demonstration has been proposed to the partner.
Demonstration of pilot-scale reactor operation	Demonstration of stable operation of pilot-scale reactors	Completed	Pilot-scale reactor operation and optimization completed.
Process optimization	Treatment capacity (as organic loading rate) reach to up to >15 kg COD/m^3/d.	Achieved	The pilot-scale An-GS has reached an OLR of ~15 kg COD/m^3 reactor/d.

4. METHODOLOGY

The project was completed in two phases, as described below.

4.1 Establishment and Optimization of Anaerobic Granular Sludge (An-GS) Reactors to Treat High-Strength DWW

Summary of work conducted. During the two-year's project, lab- and pilot-scale reactors have been operated for DWW treatment. Efforts were devoted to enhancing bioenergy (in the form of biomethane) and nutrient (e.g. phosphorus) recovery from DWW while reducing the conc entrations of contaminants (e.g. BOD, COD, pH, nutrient) of DWW to levels acceptable for sewage discharges.

4.1.1 Development of an An-GS reactor for simultaneous bioenergy and phosphorous recovery from DWW

Figure 2. Setup of the laboratory-scale An-GS reactor

Summary of work completed. Two laboratory-scale An-GS reactors were setup and operated for over 130 days under mesophilic and thermophilic conditions (Figure 2). Effective DWW treatment was achieved, featuring significant biomethane production and efficient phosphorus precipitation in the form of struvite [35].

Methods and analysis. Raw DWW (including centrate and spent caustic wastewater) was collected from Alberta Distillers, and stored at 4 °C prior to use. The centrate and spent caustic wastewater was combined to get a feasible initial pH and salinity for anaerobic digestion, and the characteristics of the mixture are shown in Table 1. Two lab-scale upflow anaerobic sludge blanket (UASB) reactors were

employed under mesophilic and thermophilic conditions to treat the DWW. The reactors were operated for over 130 days. The organic loading rates (OLRs) were set at 4.4 g COD/(L d) for Phase 1, 9.0 g COD/(L d) for Phase 2, and 18.0 g COD/(L d) for Phase 3. Correspondingly, the hydraulic retention time (HRT) of for the mesophilic and thermophilic UASB reactors were 10 days, 5 days and 2.5 days, respectively. A range of parameters, including specific methanogenic activity, microbial community structure, and treatment performance, were analyzed to assess the impact of this strategy on distillery wastewater treatment.

Table 1. Characteristics of the distillery wastewater mixture

pH	COD_{tot}	COD_{sol}	TDS	VDS	NH_4^+-N	PO_4^{3-}-P	VFA	Acetate	Propionate	Butyrate	Conductivity
	(g/L)				(mg/L)		(mg-COD/L)				(ms/cm)
6.3 (0.2)	45.4 (3.6)	40.6 (2.8)	38.5 (1.8)	28.5 (2.0)	75.8 (15.8)	236.2 (28.6)	13780 (1782)	4820 (1020)	4950 (762)	3818 (696)	15.5 (0.8)

4.1.2 Operation of an An-GS reactor for high-strength DWW treatment

Figure 3. Setup of the pilot-scale An-GS reactor

Summary of work completed. One pilot-scale reactor with a working volume of 225 liters has been setup and operated for over 130 days at the AB Distillers (Figure 3). The reactor exhibited high process stability during the entire operation, highlighting its potential for large-scale application in distillery wastewater treatment.

Methods and analysis. A pilot-scale UASB reactor, measuring 1.9 m in height and 0.4 m in diameter with a working volume of 225 liters, was utilized for the treatment of DWW. The reactor was thermally regulated using a heating blanket and insulated with polyethylene materials, maintaining internal temperatures around 30 °C. The inoculum anaerobic granular sludge, totalling 120 L, was sourced from an operational industrial full-scale UASB reactor located in Alberta, Canada. This study spanned 130 days, during which the OLRs were progressively increased from ~1 kg COD/m^3 reactor/d to ~15 kg COD/m^3 reactor/d. In tandem with the increasing OLRs, the HRT of the reactor was adjusted downwards from 5.6 days to 0.33 days. Effluent of the An-GS was collected, to be used as the feed for the A/O-GS reactor at the 2nd stage.

Raw DWW, comprising centrate, spent caustic, and process wastewater, was sourced directly from the AB Distillers. Due to the challenged conditions present in the centrate and spent caustic (e.g., pH < 4 and pH > 13, respectively), these three wastewater streams were amalgamated to achieve characteristics conducive to AD treatment. The specific characteristics of the combined wastewater, including COD, total

solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), NH_4-N, and PO_4-P, are detailed in Table 2.

Table 2. Combined distillery wastewater characteristics

Parameters	COD (g/L)	TS (g/L)	VS (g/L)	TSS (g/L)	VSS (g/L)	NH_4-N (mg/L)	PO_4-P (mg/L)
Value	5.1 ± 0.8	4.9 ± 0.9	3.6 ± 0.9	1.7 ± 0.5	0.7 ± 0.8	111 ± 37	187 ± 43

4.2 Operation of Aerobic/Anoxic Granular Sludge (A/O-GS) Reactor for DWW Post-Treatment

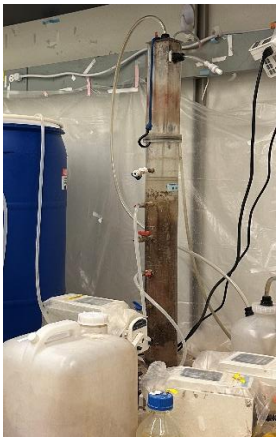

Figure 4. Setup of the A/O-GS reactor

Summary of work completed. One lab-scale GS reactor was setup and operated for over 90 days at room temperature (~21 °C) to provide DWW post-treatment for effluent of the An-GS reactor (Figure 4). The reactor demonstrated high ammonia removal of the An-GS effluent, consistently producing treated effluent meeting discharge limits.

Methods and analysis. A cylindrical A/O-GS with a 4 L capacity was employed for the treatment of An-GS effluent, which contains 150-250 mg/L NH_4^+-N, 2500–2600 mg/L alkalinity as $CaCO_3$, and 120–150 mg/L BOD. The seed sludge was granular sludge that originated from a GS reactor previously operated in Dr. Liu's laboratories. The A/O-GS reactor operated in a sequencing batch manner, with a feed exchange rate of 50% at 20 ◦C. Both feed and air were introduced from the bottom of the reactor. The reactor's operation involved 3 phases based on hydraulic retention time (HRT) and N loading. The HRT was progressively reduced from 8 h to 2 h based on the reactor capacity. Reactor's operations were automatically controlled by timers.

The reactor efficiency and stability were evaluated through comprehensive sample analyses. Influent and effluent samples were collected and filtered through 0.45 µm filter, then tested for NH_4^+-N, NO_2^--N, and NO_3^--N. Batch tests to evaluate the specific microbial activities of ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), and microorganisms responsible for denitritation and denitrification were performed in triplicate at 20 ◦C to assess the response of microbial activity to varying N loading conditions. For all the tests, the sludge samples were collected at the steady state of each stage and specifically under aerobic phase to ensure a homogeneous environment. Seed sludge and mixed liquor sludge samples gathered at the end of each stage were extracted for DNA. These DNA samples were dispatched for sequencing on the Illumina MiSeq PE250 platform and analyzed using the QIIME2 pipeline.

4.3 Further Optimization of DWW Mixing Ratios – Additional Studies

Summary of work conducted. During the earlier phases of studies, Dr. Liu's research group has identified a cost-effective approach to treat DWW, that is to mix spent caustic and acidic centrate waste streams to create suitable pH and salinity conditions for AD. In this project, additional efforts were made to confirm mixing requirements to refine process design. Of particular, the focus was to consider how to reduce the requirements for centrate addition. One strategy is to add condensate to the mixture of spent caustic and

centrate. The condensate in general has a pH similar to that of the centrate but with much lower COD and TSS concentrations; thus, is considered as a feasible alternative to centrate. A series of experiments have been conducted to pre-select the mixing ratios of centrate, spent caustic and condensate. Given the extremely high pH of spent caustic, the preferred mixing ratio should meet the pH reduction requirement, and not significantly increase the volume of wastewater to be treated. Lab-scale semi-batch reactors were operated to evaluate the pH reduction effects under different operating conditions. Mixtures of spent caustic, centrate and condensate prepared based on the pre-selected mixing ratios are used as reactor feed.

Methods and analysis. The experiment was conducted using a semi-bath mode approach at 35 °C with an HRT of 5 days. Two anaerobic sequencing batch reactors (ASBR) containing 500 mL of liquor each were employed: one without seed sludge (ASBR1) and the second one with granular seed sludge (ASBR2). The feed DWW comprised centrate, spent caustic, and condensate and its characteristics are shown in Table 3. The spent caustic was mixed with centrate and condensate to lower its pH for subsequent anaerobic digestion. The proportion of centrate in the mixture was gradually reduced with operation. A higher centrate proportion was used initially to cultivate bacteria for acidification. The lowest proportion of centrate used was when centrate, spent caustic and condensate was mixed at 0.8:1:10. pH changes and COD removal were closely monitored during the tests.

Table 3. The operation and feed characteristics for the seven stages in both ASBRs. The feed characteristics present in averages with standard deviations (in brackets). Characteristic tests were conducted at least in triplicate.

	Stage I	Stage II	Stage III	Stage IV	Stage V	Stage VI	Stage VII
Mixture (Cen:SC:Con)	4:1:10	3:1:10	2:1:10	1:1:10	1:1:10	1:1:10	0.8:1:10
HRT, d	5	5	5	5	4	3	5
pH	11.1 (0.30)	11.8 (0.20)	11.9 (0.26)	11.9 (0.25)			12.2 (0.04)
Total COD, g/L	8.71 (1.11)	7.62 (0.89)	6.36 (0.67)	4.89 (0.45)			4.57 (0.39)
Soluble COD, g/L	5.49 (0.54)	4.95 (0.39)	4.32 (0.34)	3.58 (0.26)			3.42 (0.22)
Alkalinity, mg $CaCO_3$/L	2006.67 (198.27)	2150.00 (254.33)	2315.38 (247.34)	2508.33 (226.75)			2550.85 (248.93)
Total solids (TS), g/L	15.95 (1.28)	13.11 (1.04)	9.83 (0.88)	6.00 (0.46)			5.16 (0.39)
Volatile solids (VS), g/L	4.42 (0.32)	3.82 (0.27)	3.12 (0.21)	2.30 (0.17)			2.12 (0.12)
Total dissolved solids (TDS), g/L	14.06 (1.64)	11.58 (0.99)	8.73 (0.75)	5.39 (0.38)			4.66 (0.31)
NH_4-N, mg/L	31.60 (8.65)	32.25 (6.33)	33.00 (7.24)	33.88 (8.76)			34.07 (6.46)

NO_2-N, mg/L	0.01 (0.00)	0.01 (0.01)	0.01 (0.00)	0.01 (0.00)	0.01 (0.01)
NO_3-N, mg/L	0.28 (0.02)	0.28 (0.02)	0.28 (0.01)	0.28 (0.02)	0.28 (0.03)
Total phosphorus (TP), mg/L	38.52 (5.24)	34.42 (4.39)	29.68 (3.36)	24.16 (2.73)	22.95 (2.65)
PO_4-P, mg/L	17.72 (2.29)	15.20 (2.01)	12.29 (1.56)	8.90 (1.36)	8.15 (1.12)
SO_4-S, mg/L	29.11 (5.71)	28.70 (5.58)	28.21 (4.76)	27.65 (4.93)	27.52 (4.32)
Na^+, mg/L	1339.11 (235.77)	1434.20 (321.54)	1543.92 (332.77)	1671.92 (386.26)	1700.12 (373.66)
Ca^{2+}, mg/L	23.55 (5.39)	20.28 (4.83)	16.51 (3.76)	12.11 (3.85)	11.14 (2.54)
Mg^{2+}, mg/L	18.43 (4.59)	15.11 (4.21)	11.28 (3.75)	6.81 (2.12)	5.83 (2.00)
K^+, mg/L	57.51 (8.22)	46.90 (6.71)	34.65 (5.26)	20.37 (4.56)	17.22 (3.90)
Cl^-, mg/L	22.31 (3.59)	21.15 (3.17)	19.81 (2.89)	18.25 (2.68)	17.91 (2.32)
Acetate, mg/L	1131.55 (80.63)	940.75 (81.52)	757.70 (43.43)	483.85 (0.92)	439.37 (23.18)
Propionate, mg/L	112.09 (14.71)	100.10 (19.69)	86.15 (10.32)	68.92 (9.69)	64.87 (6.25)
Butyrate, mg/L	0	0	0	0	0

4.4 Design of Integrated Processes for Full-Scale Testing

Based on the reactor optimization results from the two phases, a final evaluation of the reactor performance was conducted. A final design was formulated to integrate both GS-based processes for DWW treatment at the full-scale.

5. PROJECT RESULTS

5.1 Establishment and Optimization of Anaerobic Granular Sludge (An-GS) Reactors to Treat High-Strength DWW

5.1.1 Development of an An-GS reactor for simultaneous bioenergy and phosphorous recovery from DWW

Summary of findings. This study aimed to develop a single anaerobic GS reactor for DWW treatment while recovering both nutrients and energy, specifically in the form of phosphorus and methane. A key aspect of the study involved the implementation of a unique strategy: the mixing of centrate and spent caustic wastewater streams. This approach was intended to enhance treatment performance, manipulate the

microbial community structure, and thereby optimizing the overall treatment performance. The integration of the centrate and spent caustic streams yielded remarkable co-benefits, resulting in significant biomethane production and efficient phosphorus precipitation. The study demonstrated a phosphorus removal efficiency of ~60% throughout the 130-140 days operation period. The recovery of phosphorus via the reactor sludge offers exciting opportunities for its utilization as a fertilizer or as a raw material within the phosphorus refinery industry. The biomethane produced during the treatment exhibits significant energy potential, estimated at 0.5 GJ/(m^3 distillery wastewater) [35].

Reactor performance – Bioenergy recovery. As depicted in Table 1, the distillery wastewater exhibited high COD concentrations of 45.4 ± 3.6 g/L, a low pH of 6.3 ± 0.2, and a high conductivity of 15.5 ± 0.8 ms/cm. These characteristics pose challenges for traditional anaerobic treatment in achieving high OLRs, as they can hinder the methanogenesis activity and process stability. However, as demonstrated in Figures 5 (a) and (b), both the mesophilic and thermophilic reactors were able to consistently achieve high COD removal efficiencies throughout the three phases tested. The likely reason for the effective adaptation of the UASB reactors to these challenging conditions is attributed to the resilient micro-environment within the granules, coupled with a highly active syntrophic microbial community that is enriched in these granules.

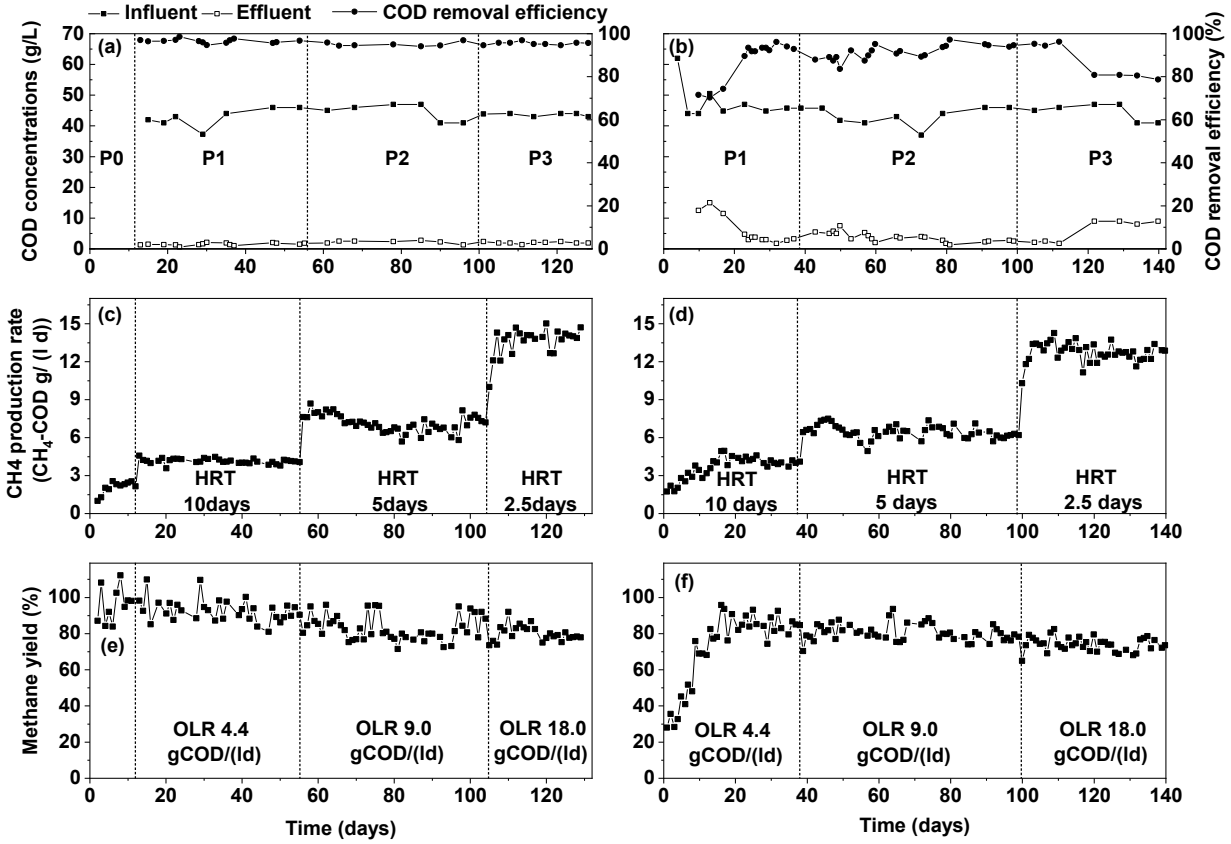

Figure 5. Performance of anaerobic DDW treatment in mesophilic and thermophilic reactors in Phases 1-3: COD removal efficiency, methane production rate and methane yield (a,c,e for mesophilic UASB reactor and b,d,f for thermophilic UASB reactor).

Specifically, the mesophilic reactor exhibited a greater COD removal efficiency as compared to the thermophilic reactor. Under all three phases, COD removal in the mesophilic reactor remained at 95.4 ± 0.9%. Even as the OLR increased from 4.4 to 18.0 gCOD/(L d) and the HRT decreased from 10 days to 2.5 days ($p < 0.05$). On the other hand, for the thermophilic reactor, COD removal efficiency was 91.9 ± 3.4% during Phases 1 and 2; however, it decreased to 86.7 ± 8.1% in Phase 3.

The decline in COD removal efficiency for the thermophilic reactor during Phase 3, when the OLR reached 18.0 g COD/(L d), can be attributed to the free ammonia inhibition under thermophilic conditions. This inhibition occurred due to the effluent pH reaching 8.5 ± 0.2 and an ammonia concentration of 1268.6 ± 115.2 mg/L. These conditions led to a significant increase in free ammonia concentration, reaching up to 330 mg/L under mesophilic conditions and 697 mg/L under thermophilic conditions. Consequently, the mesophilic reactor outperformed the thermophilic reactor in Phase 3 as OLR increased to 18.0 g COD/(L d).

The inhibition's impact, as previously discussed, is also evident in the rates of methane production, particularly within the thermophilic reactor. Figures 5 (c) and (d) depict how methane production rates increased as the HRT decreased from 10 days to 2.5 days across Phases 1-3. With an HRT of 10 days, the methane production rates were 4.1 ± 0.2 g CH_4-COD/(L d) for the mesophilic reactor and 3.7 ± 0.9 g CH_4-COD/(L d) for the thermophilic reactor. These rates rose to 7.1 ± 0.7 g CH_4-COD/(L d) and 6.5 ± 0.5 g CH_4-COD/(L d) at an HRT of 5 days, and further increased to 13.8 ± 0.7 g CH_4-COD/(L d) and 12.8 ± 0.7 g CH_4-COD/(L d) at an HRT of 2.5 days for the mesophilic and thermophilic reactors, respectively.

The resulting methane yields for the mesophilic and thermophilic reactors, as shown in Figures 5 (e) and (f), ranged from 80.5 ± 4.2% to 92.8 ± 6.3%, 74.2 ± 3.6% to 85.0 ± 5.9%, respectively, as the OLR increased from 4.4 to 18.0 g COD/(L d). These results clearly demonstrated the superiority of the mesophilic reactor in terms of methane yield compared to the thermophilic reactor for the treatment of anaerobic distillery wastewater.

The biomethane generated from this process, when quantified in energy terms, amounts to an equivalent of 0.5 GJ per cubic meter of distillery wastewater. This indicates that the methane yield from the mesophilic reactor, which has been demonstrated to be superior to that of the thermophilic reactor, could be efficiently utilized for local energy production.

Reactor performance – Phosphorous recovery. Figure 6 illustrates a substantial reduction in phosphorus within the mesophilic and thermophilic reactors. With a phosphorus concentration in raw wastewater being 236 ± 28.6 mg/L, effluent phosphorus concentration was 98.9±6.2 mg/L and 95.2±3.9 mg/L, for the mesophilic and thermophilic reactors, respectively. These represent reductions of 137.1 ± 11.5 mg/L for the mesophilic reactor, and 140.8 ± 12.3 mg/L for the thermophilic reactor, over the course of the extended operation. The removal efficiencies remained stable at 58.0 ± 2.7% and 59.6 ± 2.4% for the mesophilic and thermophilic reactors, respectively. The reduction of phosphorus consistently demonstrated similar results across the entire operation, unaffected by operational temperature. This suggests that the temperature difference between thermophilic and mesophilic operation conditions does not significantly impact phosphorus precipitation performance.

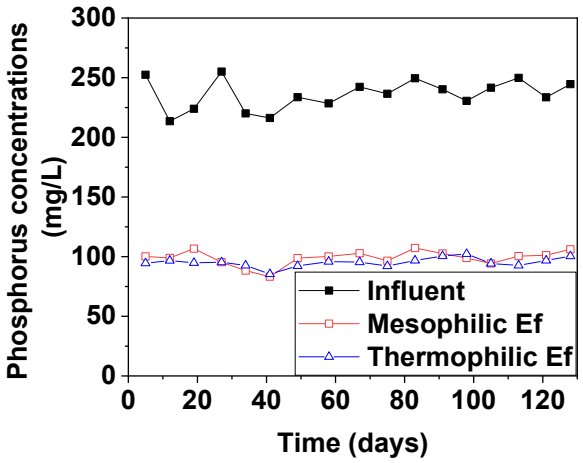

Figure 6. Phosphorus concentrations before and after anaerobic distillery wastewater treatment in mesophilic and thermophilic UASB reactors over the operation time.

The struvite supersaturation index was found to be below zero, as determined by the characteristics of the distillery wastewater. These characteristics included a pH of 6.3 ± 0.2, NH_4^+-N concentration of 75.8 ± 15.8 mg/L, and the presence of other ions (such as Ca^{2+} and Mg^{2+}) relevant to phosphorus precipitation. The Ca^{2+} and Mg^{2+} originated from the local tap water, contributing a water hardness ranging from 140-260 mg $CaCO_3$/L. The supersaturation index (< 0) indicates that phosphorus precipitation was not favorable in distillery wastewater before anaerobic treatment. Consequently, the substantial reduction in phosphorus must be attributed to mechanisms other than chemical precipitation within the anaerobic reactors. An integrated approach can be applied to enhance phosphorus recovery. This involves optimizing environmental conditions, such as pH levels, and fine-tuning chemical conditions, particularly by adjusting magnesium (Mg^{2+}) concentrations. Additionally, we suggest that operational parameters like mixing intensity play a crucial role. Adjusting these factors can significantly enhance both phosphorus recovery efficiency with the granulation process.

Sludge characterization - SMA, TSS and VSS. In Figure 7 (a), we observed that as the hydraulic retention time (HRT) decreased from 10 days to 2.5 days during Phases 1 to 3, both the mesophilic and thermophilic anaerobic reactors exhibited substantial developments in methanogenic activity. Within the mesophilic reactor, the hydrogenotrophic specific methanogenic activity (SMA) saw a modest decrease, shifting from 372.8 ± 22.8 mg CH_4 COD/(g VSS d) to 320.8 ± 16.5 mg CH_4 COD/(g VSS d). Conversely, the acetoclastic SMA experienced a more pronounced decline, dropping from 307.2 ± 21.8 mg CH_4 COD/(g VSS d) to 202.3 ± 10.6 mg CH_4 COD/(g VSS d) ($P < 0.05$). This reduction is likely attributed to the elevated levels of free ammonia inhibition, as discussed earlier.

Similarly, the thermophilic reactor witnessed significant decreases in both hydrogenotrophic and acetoclastic SMAs, with the former decreasing from 1052.0 ± 58.9 mg CH_4 COD/(g VSS d) to 526.2 ± 48.6 mg CH_4 COD/(g VSS d) and the latter from 249.2 ± 15.2 mg CH_4 COD/(g VSS d) to 82.4 ± 7.2 mg CH_4 COD/(g VSS d) ($P < 0.05$).

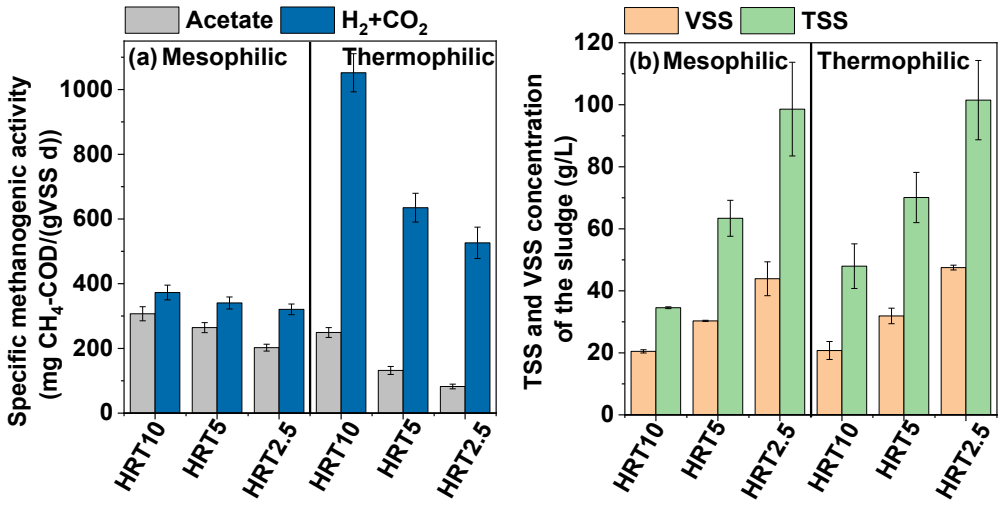

Figure 7. Profiles of the sludge in the mesophilic and thermophilic anaerobic reactors in phases 1-3 (a. SMA, b. VSS and TSS concentrations). Error bars represent one standard deviation.

Figure 7 (b) provides insight into the concentrations of volatile suspended solids (VSS) and total suspended solids (TSS) within the sludge. These concentrations increased significantly, more than doubling in the mesophilic reactor from 20.5 ± 0.5 g/L to 43.9 g/L for VSS and from 34.6 ± 0.3 g/L to 98.6 ± 15.1 g/L for TSS. In the thermophilic reactor, similar trends were observed, with VSS and TSS concentrations increasing from 20.8 ± 2.9 g/L to 47.5 ± 0.76 g/L and from 48.0 ± 7.2 g/L to 101.5 ± 12.8 g/L, respectively. These results indicate that both reactors exhibited robust biomass growth, even in the presence of challenges such as free ammonia inhibition and high salinity. This robust growth can be attributed, in part, to the self-formed struvite-like carriers (as shown in Figure 8), which enhance resilience against free ammonia inhibition and high salinity, while also increasing biomass retention within the reactors. Furthermore, the VSS/TSS ratio remained consistently low, at 0.44-0.59 and 0.43-0.47 for the mesophilic and thermophilic reactors, respectively. This low value suggests the presence of inorganic materials, likely associated with phosphorus precipitation, within the sludges.

Sludge characterization - SEM imaging. The SEM analysis of sludge from both the mesophilic and thermophilic reactors revealed striking similarities. Therefore, we focus on the SEM results from the mesophilic reactor during Phase 3, as illustrated in Figure 8.

In Figure 8 (a), we observed the lath-shaped morphology within the granules, characterized by the dimension of length 428.3 µm and width 35.0 µm, which was further identified to be struvite-like material with SEM-EDX, XRD and ICP-MS analysis in 3.3.3. These carriers provided a scaffold upon which biofilms flourished, as vividly depicted in Figure 8 (b). Notably, these images also revealed the establishment of syntrophic relationships among microorganisms, indicated by the close proximity between round and rod-shaped biomass, as seen in Figures 8 (c) and (d).

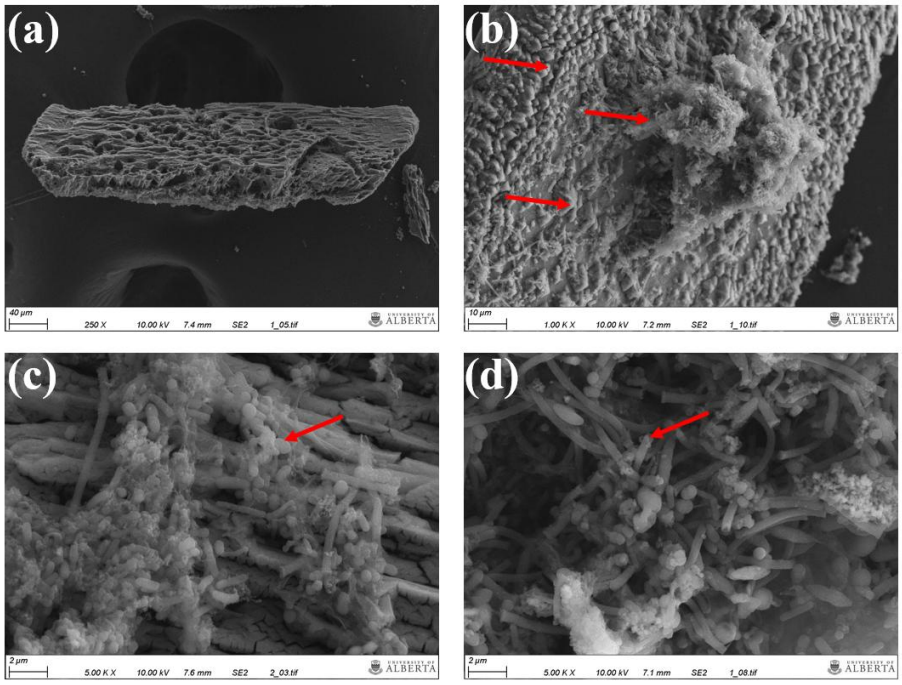

Figure 8. SEM images of the sludge collected from the mesophilic reactor at the end of phase 3 (Scale a: 40 µm, b: 10 µm, c and d 2 µm.) The red arrows were added to point out syntrophic biomass enrichment on the surface of struvite (b) and their different shapes (round (c) and rod (d)).

Sludge characterization - SEM-EDX, XRD and ICP-MS analysis. Figures 9 (a), (b), (d) and (e) present the results of SEM-EDX and XRD analyses, specifically focusing on the surface elemental composition of the granules, excluding contributions from biomass phosphorus contents, respectively for the mesophilic and thermophilic reactors. Notably, the mole ratio of magnesium (Mg) to phosphorus (P) was consistently found to be 1:1 for the two reactors, a characteristic feature of struvite ($(NH_4)Mg(PO_4 \cdot 6H_2O)$). XRD results further substantiated the presence of a struvite-like compound. Collectively, these findings confirmed that the carrier observed in Figure 8 (a) was struvite-like and the mechanism behind phosphorus removal operated in the form of struvite-like ($(NH_4)Mg(PO_4 \cdot 6H_2O)$) material.

Furthermore, ICP-MS results (Figure 9 (c) and (f)) revealed a substantial phosphorus content in the sludge, measuring 82.4 g/kg TS and 89.5 g/kg TS (total solids) for the mesophilic and thermophilic reactors, with minimal evidence of heavy metal contamination. The observed Mg:P mole ratio closely aligned with the SEM-EDX results, further supporting the identification of the struvite-like compound.

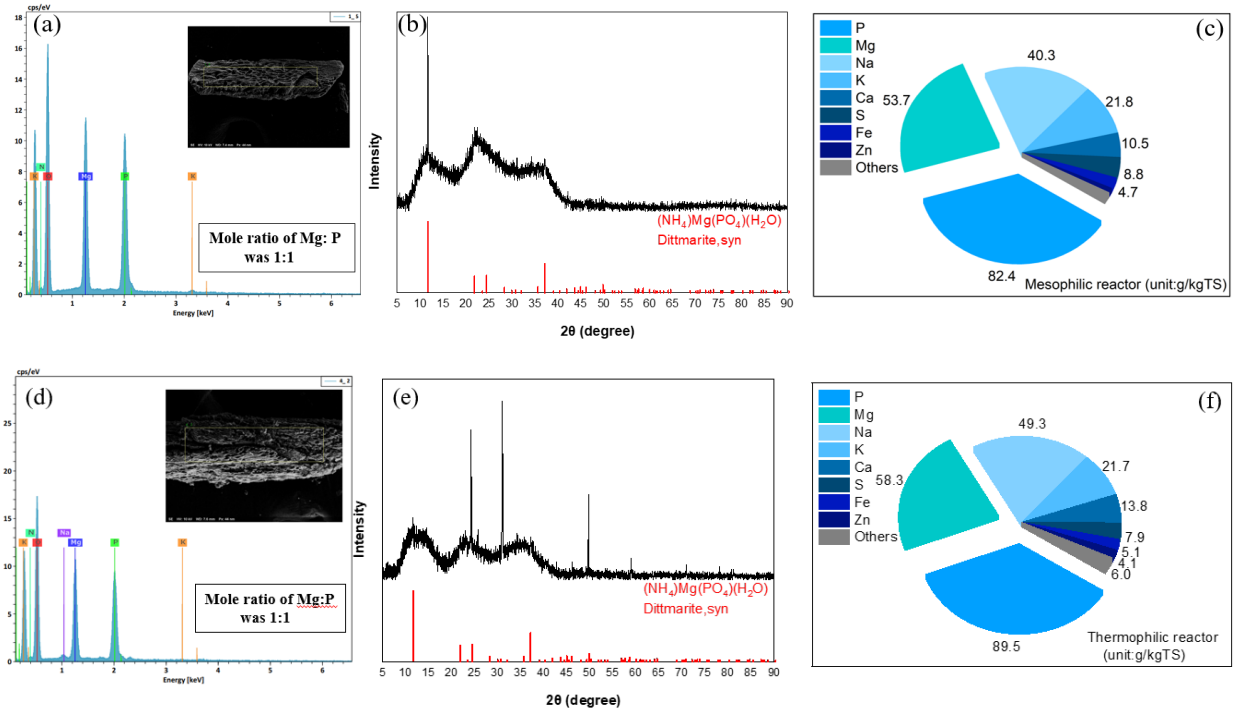

Figure 9. Characterization of the sludge from the mesophilic reactor at the end of phase 3 (a. SEM-EDX, b. XRD, and c. ICP-MS)

Microbial community analysis - Bacterial community. In Figure 10, we delved into the microbial communities within the two anaerobic reactors, the mesophilic and thermophilic reactors, across Phases 1 to 3, while operating at organic loading rates (OLR) of 4.4, 9.0, and 18.0 g COD/(L d). The relative abundances of the top 10 bacteria were characterized at both the genus and family levels (Figure 10 (a) and (b)). These results underscored the establishment of a highly functional microbial community, distinguished by its proficient fermentation and syntrophic acetate oxidation capabilities.

In the mesophilic reactor, we observed the dominance of f_Synergistaceae (8.2%) and *Syner-01* (19.5%) in the seed granules, a dominance that persisted throughout the operation, particularly in Phases 1 and 2, where their relative abundances were 10.6% and 23.4-25.9%. These microorganisms play pivotal roles in the fermentation of diverse organic materials. Notably, *Syner-01*, a representative of syntrophic acetate-oxidizing bacteria, has been previously associated with *Methanosaeta* and is known for its vital contribution to acetate reduction [36]. In our work, the formation of struvite-like carriers during anaerobic digestion likely facilitated the establishment of syntrophic partnerships by promoting close proximity among biomass. Additionally, *Thermovirga* and *W5053* saw enrichment, with their relative abundances increasing from 2.8% to 17.7% and from < 2% to 9.9% from Phase 1 to 3. *Thermovirga* is recognized for its significant role in amino acid fermentation, contributing to the degradation of deceased biomass and reducing sludge production [37].

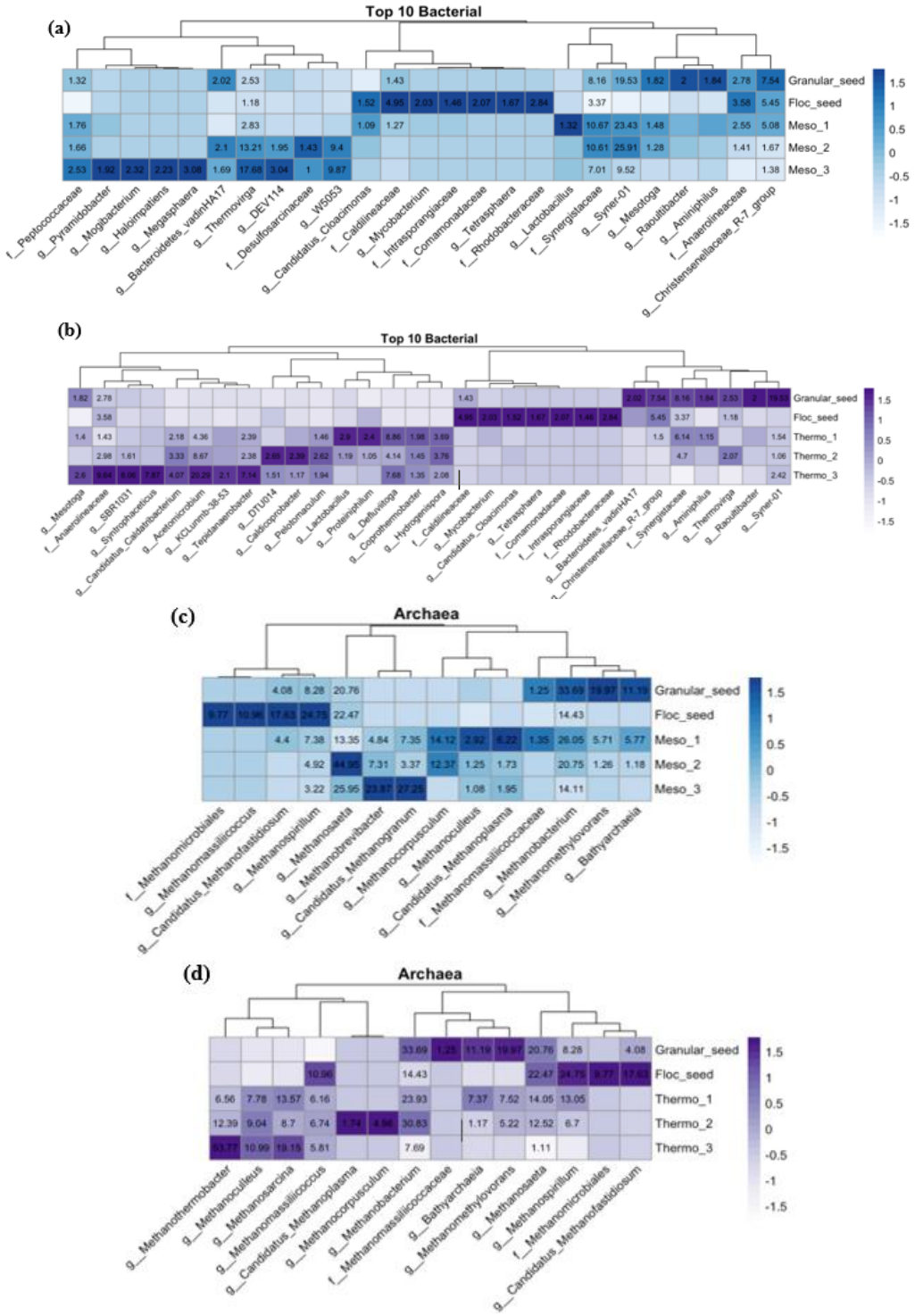

Figure 10. Microbial community of the sludge in anaerobic distillery wastewater treatment in phase 1 to 3. (a and b. Relative abundances of top 10 bacterial genera. Unidentified genera were named using the higher taxonomic levels: family (f__); c and d. Relative abundances of archaeal genera, respective for the mesophilic and thermophilic anaerobic UASB reactors). The current data set is scaled to the range of -1.5 to +1.5.

In the thermophilic UASB reactor, two bacterial members, *Defluviitoga* (4.1-8.9%) and *Acetomicrobium* (4.4-20.3%), remained dominant across Phases 1 to 3. *Defluviitoga*, a common thermophilic bacterium, is widespread and abundant, playing a central role in thermophilic biogas reactors. It is involved in the fermentation of polysaccharides, producing acetate, ethanol, hydrogen, and carbon dioxide [38, 39]. *Acetomicrobium*, another key thermophilic anaerobic bacterium, is known for generating acetate, CO_2, and H_2 from glucose [40]. These microorganisms have been reported to thrive in both propionate- and acetate-fed reactors [41], collaborating with methanogens, including *Methanoculleus*, to play essential roles in syntrophic acetate oxidation [42, 43].

In Phase 3, additional fermenters and syntrophic acetate-oxidizing bacteria took prominence, including f_Anaerolineaceae (1.4-9.6%), *SBR1031* (<2%-8.1%), *Syntrophaceticus* (< 2%-7.9%), and *Tepidanaerobacter* (2.4-7.1%). These microorganisms are integral in facilitating fermentative metabolism by utilizing carbohydrates and proteinaceous carbon sources. Specifically, *Syntrophaceticus* was identified as a putative syntrophic acetate-oxidizing bacteria candidate in thermophilic conditions [44].

In the microbial community observed, the dominant bacteria in the mesophilic and thermophilic reactors differed significantly, illustrating that operating temperature is a critical factor in shaping functional microbial groups. In the mesophilic reactor, syntrophic acetate-oxidizing bacteria from the Synergistaceae family, specifically *Syner-01*, as well as fermentative bacteria like *Thermovirga* and *W5053*, were notably abundant. Conversely, in the thermophilic reactor, fermentative bacteria such as *Defluviitoga* and *Acetomicrobium* were predominantly enriched.

Microbial community analysis - Archaeal community. Figures 10 (c) and (d) show the relative abundances of dominant methanogens in the mesophilic and thermophilic UASB reactors across Phases 1 to 3. In the mesophilic UASB reactor, two prominent methanogens, *Methanosaeta* (20.8% and 22.5%) and *Methanobacterium* (33.7% and 14.4%), maintained their dominance throughout the operation, with relative abundances ranging from 13.5% to 45.0% and 14.1% to 26.1% from Phase 1 to 3. This indicated the rapid establishment of the anaerobic distillery wastewater treatment process when using granules as inoculants. *Methanosaeta* is well-equipped for utilizing the acetoclastic pathway and contributes significantly to granule formation due to its filamentous morphology and hydrophobic properties [45]. The syntrophic bacteria effectively utilize *Methanosaeta* as a central core and structural framework for granule development. Additionally, *Methanobrevibacter* and *Candidatus_Methanogranum* were enriched, with their relative abundances increasing from 4.8% to 23.87% and 3.4% to 27.3%, respectively, from Phase 1 to 3. This diversification signified the establishment of both acetoclastic (*Methanosaeta*) and hydrogenotrophic (other dominant methanogens) pathways within the mesophilic UASB reactor. The stability and diversity of this methanogenic community reflected the robust digestion performance, characterized by effective COD removal and high methane production rates, in the mesophilic UASB reactor.

For the thermophilic UASB reactor, *Methanosaeta* (14.1% and 12.5%) and *Methanobacterium* (23.9% and 30.8%) dominated in Phases 1 and 2. However, their relative abundances dramatically decreased to 7.7% and 1.1% in Phase 3, a trend correlated with free ammonia inhibition and the high organic loading rate (OLR) of 18.0 g COD/(L d). These findings may also be linked to the observed decrease in COD removal

efficiency and methane production rate in the thermophilic reactor, particularly during Phase 3. Concurrently, *Methanothermobacter*, *Methanoculleus*, and *Methanosarcina* were enriched during the operation, with their relative abundances ranging from 6.6% to 54.8%, 7.8% to 11.0%, and 8.7% to 19.2%, respectively, from Phase 1 to 3. These thermophilic bacterial members primarily function as hydrogenotrophic methanogens and are recognized as essential partners for syntrophic acetate oxidation bacteria in acetate reduction. Their prominence in Phase 3 was instrumental in sustaining the performance of anaerobic digestion, especially as the relative abundance of *Methanosaeta* significantly decreased.

Microbial community analysis - Functional genes prediction. Within methanogens, acetoclastic methanogenesis and hydrogenotrophic methanogenesis represent the two primary pathways, with the substrates being acetate (module M00357) and hydrogen and CO_2 (module M00567), respectively. As demonstrated in Figures 11 (a) and (b), the relative abundances of genes in module M00357 and M00567 increased to 0.89% and 1.1%, and 0.40% and 0.48%, respectively, for the mesophilic and thermophilic UASB reactors. Notably, the ratio of M00567 to M00357 in thermophilic UASB reactor exhibited an increase from 0.98 to 1.21 as the OLR rose from 4.4 to 18.0 g COD/(L d). This trend underscored the growing significance of the hydrogenotrophic methanogenesis pathway.

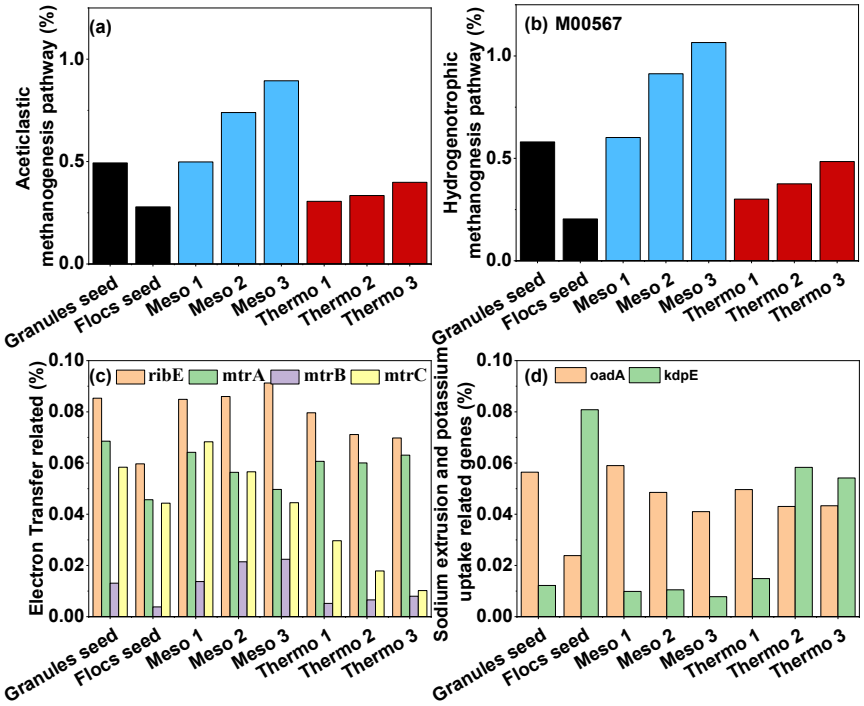

Figure 11. The relative abundance of key pathways and genes involved in the anaerobic distillery treatment as indicated by PICRUSt2 analysis, respective, for mesophilic and thermophilic UASB reactors throughout phases 1 to 3.

Genes associated with the electron transfer process, including ribE, mtrA, mtrB, and mtrC, exhibited considerable presence in both the mesophilic and thermophilic UASB reactors, as depicted in Figure 11 (c). The observed data revealed the consistent high relative abundance of these genes throughout Phases

1 to 3. The ribE gene encodes riboflavin synthetase, a key player in promoting electron transfer between extracellular c-type cytochromes and electron acceptors [46]. On the other hand, MtrA serves as a pivotal conduit for shuttling electrons from the interior to the exterior of the cell membrane, aided by the cooperative action of mtrB and mtrC [47].

Figure 11 (d) illustrates that genes associated with sodium extrusion (oadA) and potassium entry (kdpE) within the sludge of both mesophilic and thermophilic reactors maintain a consistently high relative abundance throughout Phases 1-3. Under a high salinity condition for distillery wastewater treatment, these mechanisms are crucial for preserving a balanced Na/K ratio, which is essential for maintaining cellular osmotic pressure [48]. OadA encodes oxaloacetate decarboxylase, which functions as the primary sodium pump, coupling oxaloacetate decarboxylation with the transport of sodium ions [49]. KdpE plays a crucial role in facilitating microbial cell survival under high osmotic pressure conditions. Notably, KdpE positively regulates the expression of kdpFABC, which encodes Kdp-ATPase, an inducible high-affinity potassium uptake system [50].

Application. In the context of anaerobic distillery wastewater treatment, phosphorus removal has often been overlooked or ineffective [51], necessitating additional chemical precipitation for phosphorus removal. A key significance of this study was the realization that phosphorus recovery can be concurrently achieved within the anaerobic digester while generating exceptional biofuels. The approach developed in this study simplified the treatment configuration, reduced operational and capital costs, and offered the co-benefits of improved overall performance. Importantly, this innovative process can potentially find applications in various anaerobic digestion scenarios, including the treatment of manure and waste activated sludge.

5.1.2 Operation of an An-GS reactor for high-strength DWW treatment

Summary of findings. This study demonstrated an effective anaerobic treatment of high-strength DWW at the pilot-scale. The pilot-scale reactor demonstrated stable performance at an organic loading rate (OLR) of 10.0 kg COD/m^3/day, achieving a COD removal efficiency of 86%. Average methane yield was more than 71% throughout the operation. Microbial community analysis revealed a significant increase in key syntrophic bacteria and methanogens, with *Methanobacterium* accounting for 55.8% of the archaeal population. The success of this study proved that the reactor design and treatment strategies developed by Dr. Liu's research group was effective.

Reactor performance – COD removal and methane production. Figure 12 (A) shows the COD removal efficiency of the pilot-scale reactor over the 130 days of operation. As illustrated in Figure 12 (A), the COD concentrations in the distillery wastewater ranged between 3 and 7.3 g/L, with an average concentration of 5.1 ± 0.8 g/L (Table 2). The concentrations of COD in the effluent varied from 0.3 to 3.9 g/L. Notably, within the initial 12 days, the effluent COD concentration sharply decreased from 3.9 g/L to 0.3 g/L, subsequently maintaining a stable level of approximately 0.4 ± 0.2 g/L during stages I through IV. Upon increasing the OLR to ~4.6 kg COD/m^3/day, the effluent COD concentration rose to 0.5 ± 0.0 g/L in stage V, further escalating to 0.7 ± 0.1 g/L in stage VI and reaching 0.9 ± 0.1 g/L in stage VII, corresponding with OLRs of ~10 and ~15 kg/m^3/day, respectively. It was observed that the COD removal efficiency diminished

as the OLR increased, dropping from approximately 95% to 81%. The outcomes observed in this study are in close agreement with those documented for mesophilic ADs in the literature. It was reported that mesophilic ADs typically achieve COD removal efficiencies of ~ 90% under certain conditions: when the HRT exceeds 13 hours with an OLR below 10.0 kg/m^3/d and when the HRT is more than 24 hours at OLRs greater than 13.0 kg/m^3/d [52]. In this study, when the OLR was 10.0 kg/m^3/d and HRT was around 12 hours, the COD removal efficiency was 86%. Consequently, the efficiency of this system was close to the anticipated efficiency range. Throughout the entire operation, the VSS concentration of the granular sludge in the UASB remained consistently at 53 ± 2 g/L.

Figure 12 (B) presents the methane production trends over the operation period. Initially, methane production increased from 162 ± 15 g COD/d to 514 ± 103 g COD/d across the first four stages. With a rapid increase in OLR during the last three stages, methane production significantly rose to 3085 ± 436 g COD/d at an OLR of 15 kg COD/m^3/d. During the steady state of each phase, the methane concentration in the biogas varied between 68% and 76%, while the CO_2 concentration remained at approximately 20%. The remaining components, which included limited amounts of N_2 and O_2, were present in minor quantities.

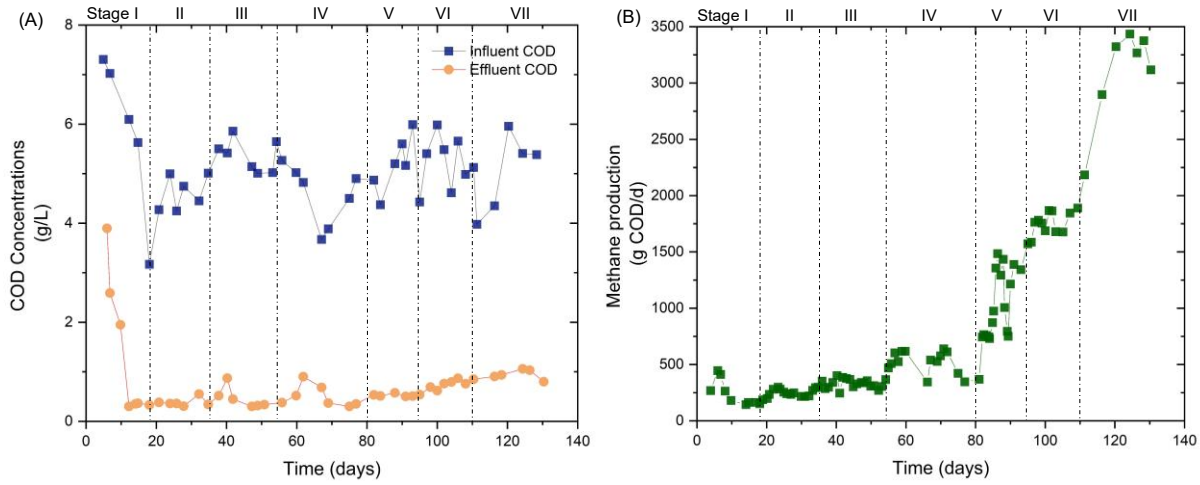

Figure 12. (A) COD removal efficiency and (B) methane production of the pilot-scale reactor

Notably, while the correlation between OLR and methane production appeared nearly linear (R^2 = 0.99), methane yields, measuring the efficiency of methane production per unit of influent COD, demonstrated significant fluctuations from 52% to over 90%, exhibiting a non-linear relationship with OLR increasing (R^2 = 0.66). These fluctuations in methane yields were likely attributed to variations in influent characteristics, including shifts in pH between 5.5 and 7 and reactor temperature variations. Although the UASB reactor was designed to accommodate the challenges posed by low pH and temperature variations, these operational factors notably influenced methane yield. Extreme conditions within the reactor may lead to the accumulation of COD, whereas optimal conditions can facilitate the consumption of accumulated COD. Overall, the average methane yield during the whole operation was more than 71%.

The high COD removal and methane production rates confirmed that blending different types of distillery wastewaters is beneficial for anaerobic digestion processes. Specifically, when the OLR was set at 10 kg

COD/m³/day, we achieved a COD removal efficiency of 86% and converted 75% of the total COD input into methane. Given that the lower calorific value of methane is 802.6 kJ/mol [53], the methane output at this OLR translated to 1.875 kg CH_4/m³/day, which is equivalent to approximately 93,904 kJ of energy per m³ of reactor volume per day. This data underscored that a cubic meter of bioreactor can produce nearly 94 MJ of energy daily, highlighting the anaerobic treatment as a highly effective and energy-efficient technology. Such efficiency not only enhances the sustainability of wastewater treatment but also contributes significantly to renewable energy generation, reflecting a compelling case for the broader adoption of anaerobic digestion technologies.

Microbial community analysis - Diversity. The Alpha diversity, represented by the Shannon and Simpson indices, demonstrated significant trends for both bacterial and archaeal communities (genus level) across various stages (Table 4). For the bacterial community, Shannon index values showed a general increase from 2.5 in the inoculum to 3.3 in stage VII, indicating a gradual enhancement in community diversity. The Simpson index similarly increased, suggesting a more equitable distribution of species as the process progressed. Contrastingly, the archaeal community exhibited a more variable pattern. The Shannon index varied, with a dip to 1.2 in stage VII, while the Simpson index displayed fluctuations and slight reductions that suggested shifts in species dominance throughout the digestion process.

Table 4. Alpha diversity of bacterial and archaeal communities

		Inoculum	S-I	S-II	S-III	S-IV	S-V	S-VI	S-VII
Bacterial diversity	Shannon	2.5	2.6	2.3	2.3	3.0	3.1	3.4	3.3
	Simpson	0.73	0.75	0.69	0.67	0.84	0.86	0.93	0.90
Archaeal diversity	Shannon	1.4	1.3	1.3	1.4	1.2	1.4	1.1	1.2
	Simpson	0.71	0.67	0.67	0.69	0.62	0.68	0.56	0.60

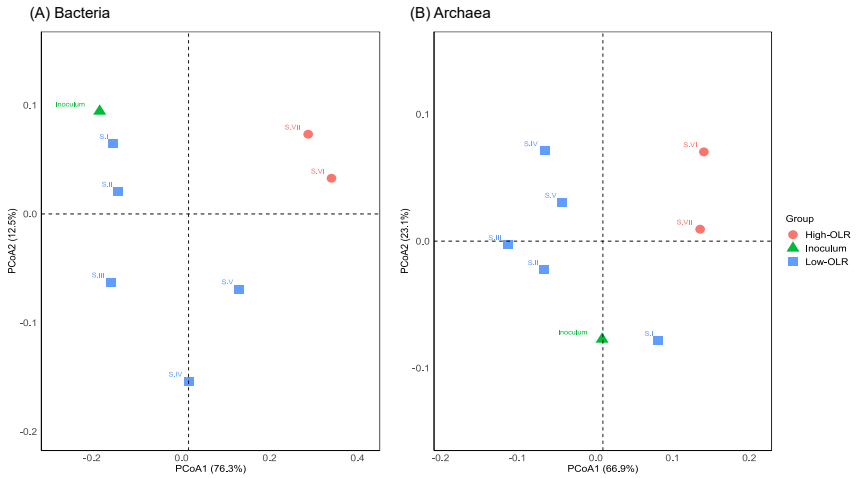

Figure 13. Principal coordinate analysis (PCoA) of bacterial (A) and archaeal (B) communities at the genus level using Bray-Curtis distances, with an OLR cutoff of 5 distinguishing high and low OLR groups.

The beta-diversity of bacterial and archaeal communities was shown through a principal coordinate analysis (PCoA) plot, employing Bray-Curtis distances among samples as shown in Figure 13(A) and (B).

Notably, both bacterial and archaeal communities demonstrated significant differentiation as the OLR increased during the operation ($p < 0.05$). Bacterial communities exhibited an obvious shift, with the Bray-Curtis distance ultimately nearing 0.5 by the operation's end, signifying considerable changes over time. In contrast, the archaeal communities followed a different pattern; despite the changes being statistically significant ($p < 0.05$), the largest Bray-Curtis distance observed among these communities was less than 0.3. This suggests that archaeal community compositions remained relatively similar throughout the operation.

Microbial community analysis – composition. Figure 14 illustrates the changes in whole microbial communities during the operation. Synergistota, initially accounting for the largest percentage of the inoculum at 55.3%, decreased to 31.0% by the operation's end. Despite its decrease, the presence of Synergistota is likely associated with the degradation of volatile fatty acids (VFAs) in the AD process [54]. Conversely, Euryarchaeota and Halobacterota increased in abundance during the operation, becoming the second and third dominant phyla, with relative abundances of 21.1% and 15.5%, respectively, by the end of the operation. Significantly, *Methanobacterium* and *Methanosaeta*, key methanogenic genera in AD, are representative of Euryarchaeota and Halobacterota respectively, illustrating the growth of methanogens during the operation [55, 56]. Furthermore, by the end of the operation, Desulfobacterota and Bacteroidota saw an increase in their relative abundance to 5.9% and 5.7%, respectively, ranking them as the fourth and fifth most prevalent phyla. Many genera within Desulfobacterota function as syntrophic/sulfate-reducing bacteria, while those within Bacteroidota might be associated with fermentation [57]. These microorganisms are pivotal in the AD, highlighting their essential roles in the complex microbial ecosystem responsible for effective waste breakdown and biomethane production.

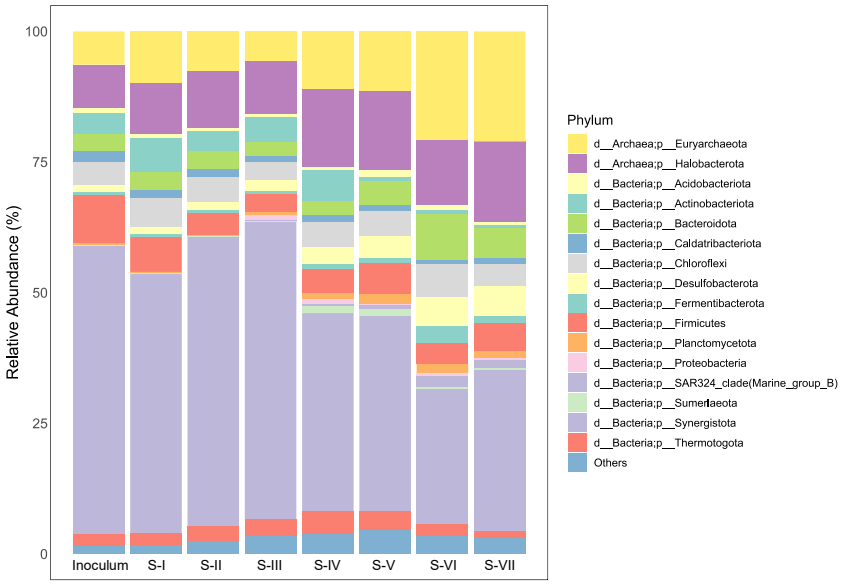

Figure 14. Microbial community composition at phylum level.

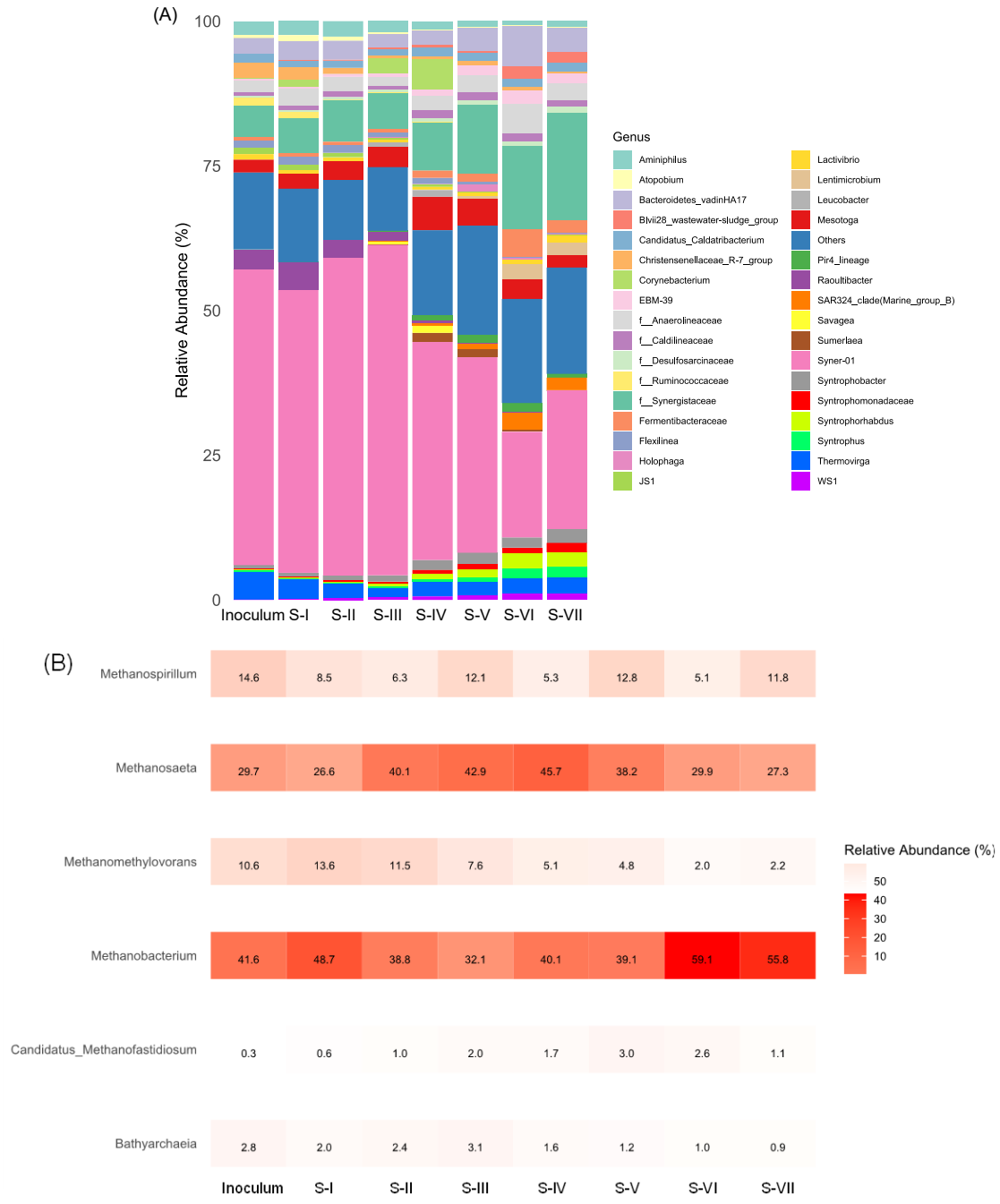

Figure 15. The relative abundances of bacterial genera (A) and archaeal genera (B) with average abundance >1% in the inoculum, and different stages.

Figure 15 (A) illustrates the changes in the bacterial community at the genus level. A significant shift in the microbial communities was observed. *Syner-01*, a genus within the Synergistota phylum, initially dominated the inoculum with a relative abundance exceeding 51.2%. As the AD process progressed, the abundance of *Syner-01* declined, eventually stabilizing at 23.8% by the end of the operation, yet it remained the most dominant genus. Simultaneously, an unidentified genus from the *Synergistaceae*

family, closely related to *Syner-01*, experienced a growth from 5.6% to 18.6%, emerging as the second most dominant genus by the conclusion of the operation. Given that *Synergistaceae* is potentially associated with syntrophic acetate oxidation (SAO) [58, 59], the prominent presence of both *Syner-01* and the unidentified genus from *Synergistaceae* hinted that the SAO pathway might be one of the important pathways in the AD of distillery wastewater. *Bacteroidetes_vadinHA17* emerged as the third most dominant genus, achieving a relative abundance of 4.3%. This genus is potentially characterized by a facultative metabolism, playing a pivotal role in the breakdown of complex organic carbon compounds [60]. The collective relative abundances of syntrophic bacteria, specifically *Syntrophorhabdus*, *Syntrophobacter*, *Syntrophomonadaceae*, and *Syntrophus*, experienced an increase throughout the study [61, 62]. Although each genus individually accounted for a small proportion, together, their total abundance rose from 1.1% to 8.3%. This significant increase underscored the crucial role of syntrophic relationships in the anaerobic digestion system utilized for treating distillery wastewater.

Figure 15 (B) illustrates the shifts within the archaeal community. *Methanobacterium*, the most abundant genus within the inoculum at 41.6%, saw its relative abundance increase to 55.8% by the end of the operation. *Methanosaeta*, initially the second most abundant genus, experienced a slight decrease from 29.7% to 27.3% during the operation, while *Methanospirillum*'s presence diminished from 14.6% to 11.8%. The increase in *Methanobacterium* suggested its robust capacity for methane production under the operational conditions of this study, possibly indicating a shift towards hydrogenotrophic methanogenesis as a dominant pathway [63]. The slight decrease in *Methanosaeta* suggested that acetoclastic methanogenesis remains a crucial process, despite shifts in community dynamics [64]. These changes highlighted the dynamic of the archaeal community in the AD of distillery wastewater treatment.

Relationships between operational factors and microbial community analysis. The Mantel test results, as presented in Table 5, demonstrated significant relationships between operational factors and microbial community compositions in the UASB reactor during the treatment of distillery wastewater. The overall Mantel score for all factors combined is 0.7316 ($p = 0.002$), indicating a strong correlation between operational conditions and microbial community structure. Among the individual factors, OLR showed the highest Mantel score ($r = 0.7342$, $p = 0.004$), highlighting a particularly strong association between OLR and shifts in the microbial community within the reactor. Distillery wastewater is typically highly biodegradable [23], and as OLR increases, VFA concentrations in the UASB may also rise, fostering syntrophic relationships between bacteria and methanogens.

Table 5. Mantel correlations highlight the relationships between operational factors and microbial community compositions in the pilot reactor

Operational factors	Mantel score (r)	P-value
All the factors	0.7316	0.002
Influent COD	-0.1044	0.644
Influent pH	0.0099	0.471
OLR	0.7342	0.004
HRT	0.3769	0.049

As shown in Figure 15 (A), the relative abundance of syntrophic bacteria increased from 1.1% to 8.3% in response to rising OLR. Additionally, the increase in *Methanobacterium*, a hydrogenotrophic methanogen, was likely related to higher OLR, as it efficiently utilizes the hydrogen and CO_2 produced during VFA degradation. These syntrophic interactions are crucial for the efficient breakdown of VFAs, a key process in the anaerobic digestion of high-strength wastewater like distillery effluent.

Application. Given the biodegradability of distillery wastewater, the primary challenge in anaerobic treatment of distillery wastewater should focus on enhancing syntrophic relationships between VFA-oxidizing bacteria and methanogens to maintain process stability under high organic loading conditions. Findings from this study suggested that optimizing syntrophic interactions between key microbial groups is crucial for further enhancing reactor performance in high-strength distillery wastewater treatment. The use of conductive materials, such as granular activated carbon, could enhance electron transfer and strengthen syntrophic relationships [65], offering a promising approach for optimizing distillery wastewater treatment for future study.

5.2 Operation of Aerobic/Anoxic Granular Sludge (A/O-GS) Reactor for DWW Post-Treatment

Summary of findings. This study demonstrated an effective post-treatment of the An-GS treated DWW. With an HRT of 2 hours, the A/O-GS achieved high ammonia removal efficiency (>99%), demonstrating extremely high treatment capacity at a relatively small foot print.

Reactor performance. Figure 16 shows the ammonia removal of the A/O-GS reactor. As shown in Figure 16, the reactor was successfully started up after ~5 days of operation at an HRT of 8 hours and maintained stable operation in Phase 1, reaching an ammonia removal of >99%. In Phase 2, reduced ammonia removal was observed during the first 2 days (Day 35-36) of operation possibly due to significantly increased ammonia loading rate caused to sharply reduced HRT from 8 hours to 3 hours. However, the reactor showed rapid recovery from Day 37, and again reached over 99% ammonia removal from Day 40. Further reducing the HRT to 2 hours did not affect the reactor's performance regarding ammonia removal. During the entire operation, SVI_{30} was consistently below 30 mg/L, indicating the formation and presence of granular sludge.

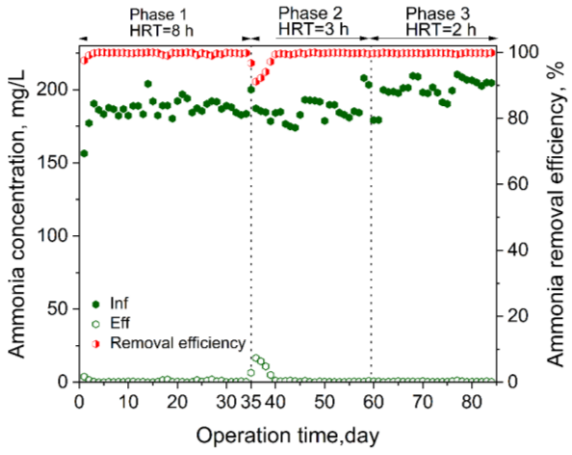

Figure 16. Ammonia removal of the A/O-GS reactor

Microbial kinetics. Specific microbial activities of AOB, NOB, denitritation and denitrification are shown in Figure 17. Both AOB and NOB activities increased with reduced HRT. The AOB activities as shown in Figure 17a had the lowest value in Phase 1 at 0.25 g N/g VSS/d then increased with shortened HRT and reached the highest value in Phase 3 at 0.84 g N/ g VSS/d, which fitted at the high end of the reported AOB activity values in literature. The NOB activities were the lowest in Phase I at 0.18 g N/g VSS/d, and peaked at 0.89 g N/g VSS/d in Phase 3. It was noted that at the end of Phase 2, NOB activities became much higher than AOB activities, suggesting high rate of nitrite oxidation in the reactor. Cycle test results at the end of Phase 2 showed low nitrite accumulation during aeration, further proving the rapid development of NOB activities.

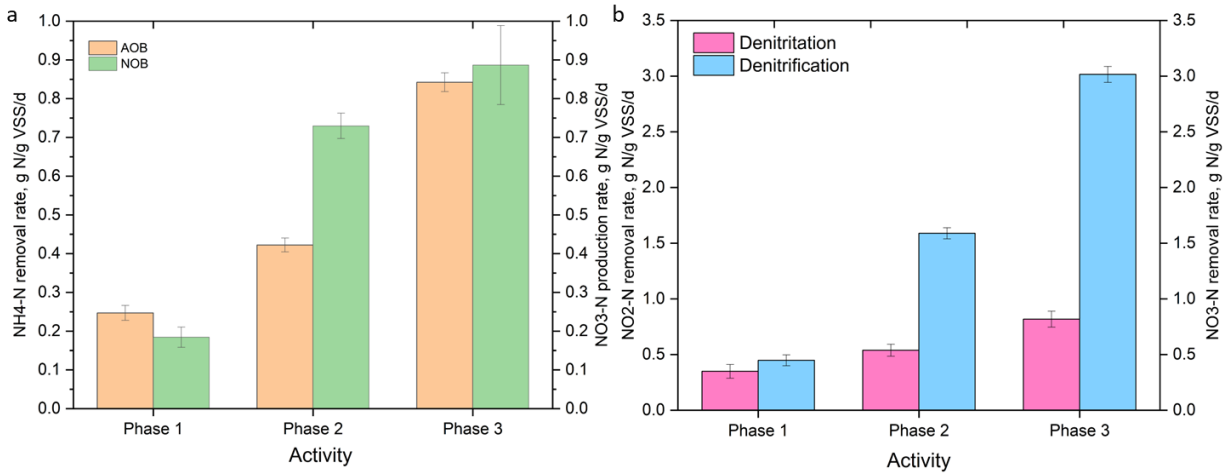

Figure 17. Specific microbial activities under each stage, including a) the specific activities of ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB); b) the specific activities of denitritation and denitrification.

The denitritation and denitrification specific activities were shown in Figure 17b. The denitritation activities increased slightly from 0.35 to 0.82 g N/g VSS/d from Phases 1 to 3. In contrast, the denitrification activities demonstrated rapid increase from 0.45 to 3.02 g N/g VSS/d, which was 4-fold higher than the highest denitritation activities. These results suggest that increased N loading also enhanced the denitrification activity in the A/O-GS.

Microbial community analysis. Overall, reactor operation led to notable shifts in bacterial composition across different phases (Figure 18). Microbial community analysis revealed a consistent enrichment of *Thauera*, *Nitrosomonas*, *Paracoccus* and *Chryseolinea* throughout reactor operation, suggesting the development of highly functional microbial community for ammonia and organics removal under the operational conditions. Among them, *Thauera* and *Paracoccus* are widely known as denitrifying bacteria, *Nitrosomonas* are well-recognized ammonia-oxidizing bacteria (AOB), and *Chryseolinea* are characterized by their ability to degrade organics. *Chryseolinea* have also recently been proven to be aerobic denitrifying bacteria [66], and are beneficial for maintaining the stability of granules [67]. Enrichment of these bacteria highlighted the development of conventional nitrogen removal pathways in the reactor. During conventional nitrogen removal, autotrophic AOB and nitrite-oxidizing bacteria (NOB) convert NH_4^+-N to NO_3^--N, followed by denitrifying bacteria to reduce NO_3^--N to N_2.

Further, recent studies started to highlight the importance of heterotrophic nitrification-aerobic denitrification (HN-AD) in wastewater nitrogen removal and the significance of *Paracoccus* in HN-AD [68]. Different from conventional nitrogen removal pathway, during HN-AD, nitrification and denitrification both occur within the single cell of a bacterium, achieving simultaneous nitrogen removal [69]. This allows for complete nitrogen removal in one aerobic reactor [70]. The significant enrichment of *Paracoccus* as well as the largely increased NOB activities throughout the three phases of operation suggested the possibility of co-development of the HN-AD pathway in the reactor.

Specifically, as shown in Figure 18, the seed sludge was dominated by *Hyphomicrobium* (27.4%, heterotrophic denitrifying bacteria), followed by *Thauera* (9.2%, heterotrophic denitrifying bacteria), *Nitrosomonas* (6.9%), *Truepera* (5.3%, autotrophic denitrifying bacteria), nitrite-oxidizing Chloroflexi *G30-KF-CM66* (4.1%), *f_Comamonadaceae* (2.6%, heterotrophic denitrifying bacteria), *Limnobacter* (2.4%, denitrifying bacteria), nitrite-oxidizing Chloroflexi *AKYG1722* (2.4%), nitrite-oxidizing Chloroflexi *OLB14* (2.2%) and P*haeodactylibacter* (2.0%, denitrifying bacteria). For these dominant genera (≥ 2%), the relative abundance of AOB, NOB and denitrifiers in seed sludge were 6.9%, 8.7% and 48.9%, respectively.

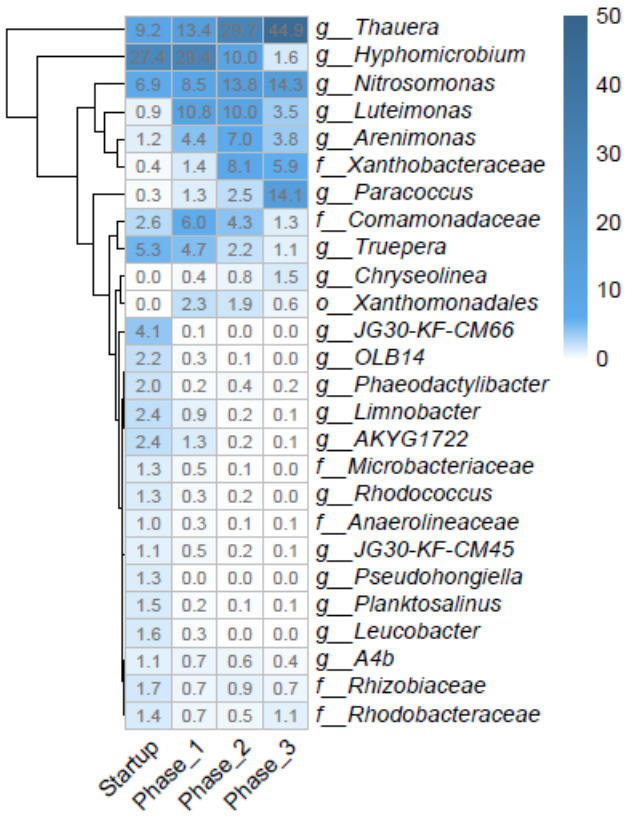

Figure 18. The relative abundances of bacterial genera of A/O-GS with average abundance >1% in the inoculum, and different phases

After Phase 1, *Hyphomicrobium* (29.4%), *Thauera* (13.4%), *Luteimonas* (10.8%, heterotrophic nitrite reducing bacteria), *Nitrosomonas* (8.5%), *f_Comamonadaceae* (6.0%), *Arenimonas* (4.4%, denitrifiers),

o_Xanthomonadales (2.3%, hydrocarbon degraders), *f_Xanthobacteraceae* (1.4%, organic degrading bacteria), *Paracoccus* (1.3%), and *Chryseolinea* (0.4%) showed enrichment compared to the seed sludge, while other genera (e.g. *Truepera* and nitrite-oxidizing Chloroflexi *G30-KF-CM66*) exhibited a declining trend.

Reducing the HRT to 3 hours and eliminating the anoxic recirculation step in Phase 2 continuously enriched *Thauera* (29.7%), *Nitrosomonas* (13.8%), *Arenimonas* (7.0%), *f_Xanthobacteraceae* (8.1%), *Paracoccus* (2.5%), and *Chryseolinea* (0.8%); however, the relative abundance of other previously enriched genera (e.g. *Hyphomicrobium*, *Luteimonas*, *f_Comamonadaceae,* and *o_Xanthomonadales*) started to decline, comparing to the end of Phase 1. It was reported previously that low C/N ratio favors the growth of *Thauera* over other competitors in low-carbon wastewater [71]. This may have contributed to the increased *Thauera* and reduced *Hyphomicrobium*. It was worth noting that although the relative abundance of NOB (e.g. Chloroflexi *G30-KF-CM66*, *AKYG1722*, *OLB14*, and *A4b*) experienced decrease in Phase 2, the NOB activities still showed rapid increase (Figure 17a), highlighting the possible establishment of the heterotrophic nitrification pathway in the reactor.

In Phase 3, with the HRT further reduced to 2 hours, *Truepera* (44.9%), *Nitrosomonas* (14.3%), *Paracoccus* (14.1%), and *Chryseolinea* (1.5%) showed significant increases, whereas most other species experienced population reductions compared to Phase 2. However, *Luteimonas* (3.5%), *Arenimonas* (3.8%), *f_Xanthobacteraceae* (5.9%), and *o_Xanthomonadales* (0.6%) still remained enriched relative to the seed sludge, demonstrating the impacts of operational conditions on A/O-GS community dynamics.

Application. The A/O-GS system demonstrated effective post-treatment of DWW following anaerobic digestion, achieving over 99% ammonia removal and subsequent COD reduction. This further improved the quality of treated DWW, helping large industrial distillery wastewater dischargers comply with regulatory standards.

5.3 Further Optimization of DWW Mixing Ratios – Additional Studies

Summary of key findings. This study demonstrated the feasibility of anaerobically digesting highly alkaline spent caustic by blending it with acidic centrate and condensate. The optimal operating conditions identified were a centrate, spent caustic, and condensate mixture ratio of 1:1:10 and an HRT of 3-5 d. Under these conditions, a feed pH value of 11.9 was reduced to approximately 8.0 in the effluent, over 90% COD was removed, and about 75.9% of the removed COD was converted to biomethane.

pH changes. Figure 19 shows the changes in effluent pH for both ASBRs across the seven stages. ASBR1, the control reactor, was not inoculated. In Stage I (Cen:SC:Con=4:1:10), the effluent pH gradually decreased from 10.7 to 6.6, with floc formation observed during this initial stage. In Stage II (Cen:SC:Con =3:1:10), with an increased proportion of spent caustic, the effluent pH initially rose to 8.4 before gradually decreasing to 6.8, and the flocs developed into small amounts of sludge. During Stage III (Cen:SC:Con =2:1:10), the effluent pH continued to increase, reaching 11.3, which led to reactor deterioration as the formed sludge was unable to survive under such a highly alkaline condition.

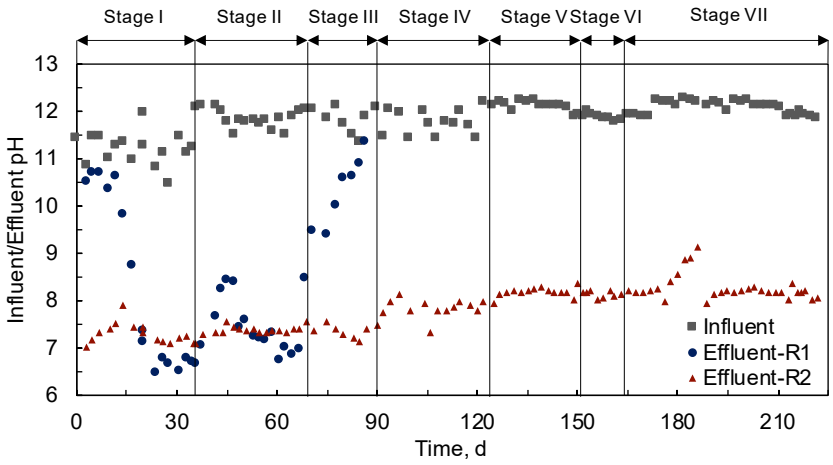

Figure 19. Influent and effluent pH values during the seven stages. ASBR1 deteriorated in Stage III

The operation of ASBR2, which was inoculated with anaerobic granular sludge, showed distinct pH trends across the seven stages with varying mixing ratios of centrate, spent caustic, and condensate (Figure 19). From Stage I to Stage VII, the influent pH increased from 11.1 ± 0.3 to 12.1 ± 0.1. Correspondingly, the effluent pH remained relatively stable, with values of 7.3 ± 0.3, 7.4 ± 0.1, 7.4 ± 0.1, 7.8 ± 0.2, 8.2 ± 0.1, 8.1 ± 0.1, and 8.3 ± 0.3 for each stage, respectively. This stable and low effluent pH in ASBR2 throughout the operation indicates that the microbial community successfully adapted to higher spent caustic levels. In contrast to ASBR1, the granular sludge inoculation in ASBR2 enabled the microbial consortia to develop resilience to variations in influent composition, highlighting the importance of granular sludge seeding for maintain stable and effective reactor operation.

COD changes. Figure 20 shows changes in COD, including total COD (tCOD) and soluble COD (sCOD), from feed to effluent. As the proportion of spent caustic in the influent increased, ASBR1's tCOD removal efficiency rose from 56.1% in Stage I to 68.8% in Stage II, but then declined to 33.1% in Stage III. Similarly, ASBR1's sCOD removal efficiency improved from 68.6% in Stage I to 81.1% in Stage II before dropping to 37.4% in Stage III. Without inoculation, the microbial community in ASBR1 was not well-developed, making it unable to manage fluctuating influent compositions effectively.

In contrast, ASBR2 consistently achieved lower effluent tCOD and sCOD levels. From Stage I to Stage IV, tCOD removal exceeded 90%. However, when the HRT of ASBR2 decreased from 5 d to 3d (Cen:SC:Con=1:1:10), tCOD removal efficiency dropped from 91.3% to 73.1%. At the highest proportion of spent caustic in Stage VII (Cen:SC:Con=0.8:1:10), tCOD removal further decreased to 65.5%. Nevertheless, sCOD removal remained above 90% throughout the operation. These results confirm that with granular sludge inoculation and a mixing ratio of 1:1:10 of centrate, spent caustic and condensate, a more effective microbial community could be established, allowing for enhanced COD removal and system stability.

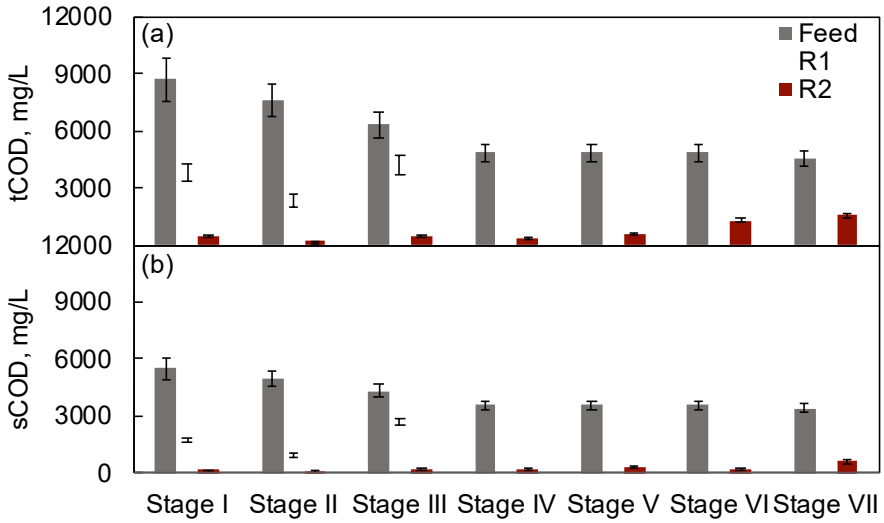

Figure 20. Average total COD and soluble COD concentrations in feed, R1 effluent, and R2 effluent during the stable operation for the seven stages

5.4 Design of Integrated Processes for Full-Scale Testing

Evaluation of overall process efficiency. The integrated An-GS and A/O-GS processes developed in this project achieved cost-effective BOD and ammonia reduction (Table 6). Notably, the treated DWW had a BOD of approximately 40 mg/L, well below the Wastewater Bylaw limit.

Table 6. Key parameters of treated distillery wastewater

	Raw DWW	An-GS treated DWW	A/O-GS treated DWW	Overall removal efficiency (%)
pH	6.0-7.1	8.0-8.2	7.9-8.3	-
BOD, mg/L	2,600-3,100	120-150	20-45	>98
NH_4-N, mg/L	98-148	150-250	<1.0	>99

Design of Integrated processes. Based on the operation of the An-GS and A/O-GS reactors, as well as the optimized mixing ratios of various DWW streams, an integrated treatment process was proposed to the industrial partner. The proposed system is designed to treat 10–13 m^3/day of DWW with a mixing ratio of 1:1:10 for spent caustic, centrate, and condensate, respectively, at an HRT of 0.6 days. The process includes two mesophilic An-GS reactors (2.5 m diameter × 8 m height) and two A/O-GS reactors (1.6 m diameter × 5.5 m height), completing the two-stage treatment (Figure 21).

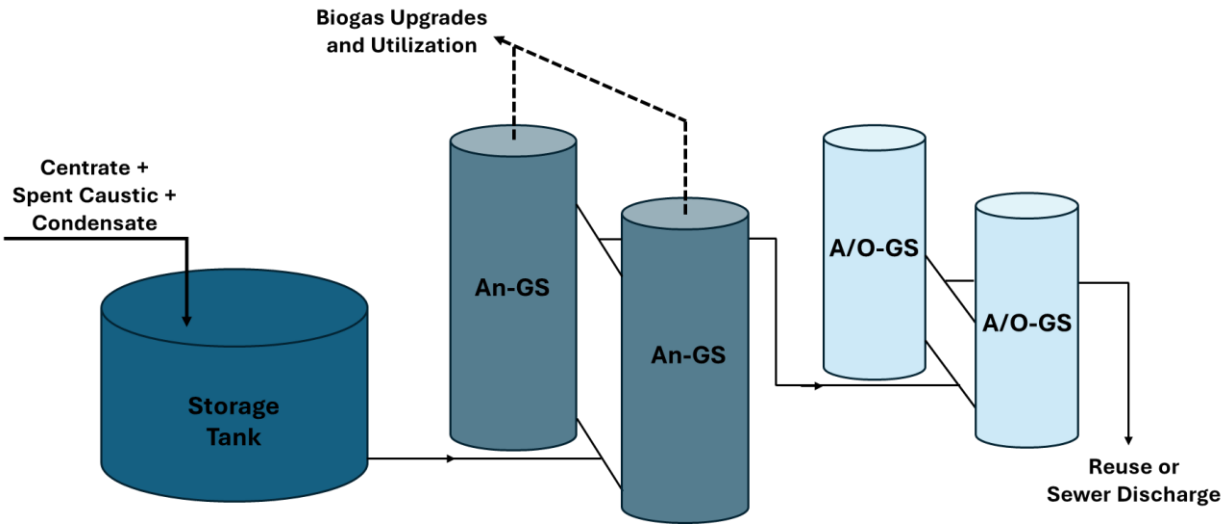

Figure 21. Schematics of proposed full-scale design (not to scale)

6. KEY LEARNINGS

The key takeaway from this project is that the integrated anaerobic granular sludge (An-GS) and aerobic/anoxic granular sludge (A/O-GS) process offers a cost-effective and robust solution for treating high-strength distillery wastewater, as validated through extensive laboratory and pilot-scale studies. Laboratory-scale An-GS experiments demonstrated the advantages of co-digesting centrate and spent caustic streams, achieving simultaneous and substantial biomethane production alongside efficient phosphorus precipitation. The recovery of phosphorus through reactor sludge presents promising opportunities for reuse, either as a fertilizer or as a raw material in the phosphorus refining industry.

Pilot-scale operation of the An-GS reactor further confirmed the effectiveness of the reactor design and treatment strategies developed by Dr. Liu's research group. Over a continuous 130-day period, the pilot reactor exhibited stable performance at an OLR of 10.0 kg COD/m^3/day, consistently achieving a COD removal efficiency of 86% and an average methane yield exceeding 71%. Microbial community analysis revealed a significant enrichment of syntrophic bacteria and methanogens, with *Methanobacterium* comprising 55.8% of the archaeal population, indicating the successful establishment of a highly efficient anaerobic digestion microbial consortium.

The integration of the A/O-GS process with the An-GS system further enhanced effluent quality, yielding overall BOD removal above 98% and ammonia removal exceeding 99%, thereby meeting stringent sewer discharge regulations.

This project addresses a major challenge facing water-intensive distillery operations: reducing water consumption and minimizing wastewater generation while complying with discharge standards. The findings provide valuable insights for the adoption of high-rate treatment technologies in the distillery sector, supporting improved energy efficiency, resource recovery, and opportunities for water reuse.

7. OUTCOMES AND IMPACTS

7.1 Project Outcomes and Impacts

This project extensively investigated BOD and ammonia removal from high-strength distillery wastewater, systematically exploring the underlying mechanisms. It successfully established an integrated An-GS and A/O-GS process at the laboratory scale and demonstrated stable pilot-scale operation over ~130 days. The integrated process achieved over 98% BOD removal and 99% ammonia removal within a short hydraulic retention time (HRT) of 0.6 days, supporting a high organic loading rate (OLR) of 15 kg COD/m^3 reactor/day. These results highlighted the strong potential of the An-GS and A/O-GS process for treating high-strength distillery wastewater and other similarly characterized wastewaters.

7.2 Clean Energy Metrics

Please see Section 3.3.1 for the clean energy metrics.

7.3 Program Specific Metrics

Please see Section 3.3.2 for the program specific metrics.

7.4 Project Outputs

Please see Section 10.2 for journal articles and conference presentations.

8. BENEFITS

8.1 Economic Benefits

The proposed innovation is a highly effective organics and nutrients removal alternative to treat DWW at a relatively low cost. There are several direct economic benefits to the industry: i) Reduce the cost of water and chemical required to reduce DWW contamination and to meet sewer discharge standards; ii) Improving process safety measures by eliminating strong acid/based handling for pH adjustment; iii) Reduce the surcharge costs associated with DWW discharging into the sewer system and sludge disposal thanks to improved treatment efficiencies; iv) Reduce power bills when biogas is recovered and used as an alternative heating source; and v) improve AB Distillers' environmental performance through water reuse and bioenergy/phosphorus recovery.

The success of this project provided an immediate commercialization opportunity. Discussion has been initiated for the establishment of a demonstration site at Alberta Distillers' Calgary facility. This will create job opportunities for contractors, builders, operators and engineers. It is also foreseeable to attract additional investment from both food and beverage industries with the need and desire to better manage their wastewater to reduce cost and environmental footprint.

8.2 Environmental benefits

8.2.1. Water benefits

The distillery industry in Canada is water intensive. In Canada, ethanol (i.e., alcohol in drinks, gasolines, etc) production in Canada was about 1.8 billion litres per year from 2013 to 2017; whiskey sales reached approximately 48.35 million litres per year (2019 to 2020) [51] and approximately 171,210 kL distilled spirits [52] were exported from Canada in 2020 (CAD$781.2 million) [53]. For every litre of alcohol produced, molasses-based distilleries consume 14-22 L water for process applications such as yeast propagation, molasses preparation, and steam generation, 100-240 L water for non-process applications such as steam generation, cooling, and cleaning; and generate about 8-15 L of wastewater [1]. In Canada, there are 354 distillers [2] and the volume of DWW generated is estimated to be ~ 0.74 million m^3/day [2, 3]. This number is significant comparing to an average of 16.6 million m^3/day of municipal wastewater discharged in Canada [5].

The immediately and most significant environmental benefit is the water benefit. The innovation could help significantly reduce water consumption and wastewater generation. Separate treatment of the low-strength and high-strength DWW increases the reuse potential of non-process DWW. The high-rate high-efficiency process can effectively remove the pollutant loads in DWW, thus eliminating the needs to dilute DWW before discharging, hence saving water and reducing wastewater volume. Particularly in Alberta, Alberta Distillers, as one of the oldest whisky distilleries in Canada, is the largest distillery in the province [24]. It is one of the top eight Canadian whiskey producers that together produce over 95% of the whiskey in Canada [54]. Hence, the DWW footprint of Alberta Distillers is huge and even leading in Canada. The project aligned with Alberta Distillers' desire to set up their own inhouse fit-for-purpose DWW treatment, and could help them reduce them wastewater discharges into the City's sewer collection system, which in alternative, helps the City reduce their wastewater treatment burden. The development of technologies for cost-effective DWW treatment and resource recovery with Alberta Distillers will benefit Alberta in providing clean water with much reduced environmental footprint, and support Alberta's ongoing efforts to fight climate change. Such treatment is also applicable to other distillery industries and to other water intensive industries, such as food processing, agriculture, and diary industries.

8.2.2. GHG benefits

The proposed technology features energy-efficient AD process for biogas recovery and phosphorous recovery, as well as an all-in-one energy-efficient biological process for ammonia reduction, all of which are beneficial for greenhouse gas (GHG) reduction. The adoption of the dynamically controlled granular sludge based process features high-density and high-activity microbial biomass cultivation, hence, the innovation only produces a very low amount of organic biosolid, which otherwise needs a separate process for dewatering and disposal with additional cost.

8.3 Social benefits

Evaluating and optimizing industrial wastewater treatment and resource recovery is not unique to Alberta. There is a worldwide need for expertise in this area and a great deal of capacity for highly qualified people trained in this area. From this project, researchers and employees from the Alberta Distillers were

trained to operate comfortably and knowledgeably in the areas of DWW analysis and treatment, reactor operation and optimization. Their skillset should prove invaluable, as work in these areas must advance if the nations' waste services are to be protected and improved.

The shortage of workers qualified to manage high-strength wastewater was addressed in this project. Trainees obtained academic and industrial knowledge and experience that will ease their transition to university or corporate positions. Canadian universities and industries would reap the benefits of the personal expertise fostered by this project. Development of this expertise will not be limited to use in the treatment of distillery, brewery and winery wastewater in Canada, but can be applied to the treatment of other high organics and high nutrients wastewater around the world. Having the expertise and the trained workforce in Canada will provide new markets for Canadian companies in the international markets. The Province of Alberta has recognized through various planning strategies and frameworks the critical importance of improving wastewater treatment to protect public and environmental health - for example the emphasis in the Water for Life strategy on source water protection. The project provided an innovative and holistic solution regarding credible alternatives for wastewater management, further developing Canada (and Alberta) as a hub for environment-focused sustainable development.

Further, the value of Equity, Diversity, and Inclusion (EDI) is also emphasized. To promote EDI and overcome the issues of male dominance in engineering and underrepresented groups, we implemented EDI best practices in the recruiting. The positions were advertised broadly for at least 30 days using unbiased, ungendered language. Before any selection, we developed objective, performance-based evaluation criteria. During recruitment, we blinded unnecessary information (e.g., gender, age, and ethnicity) and adhered to the evaluation criteria. With our efforts, we encouraged more women and underrepresented groups to be engaged in the project and reached successful outcomes, serving as visible representatives for others.

8.4 Building innovation capacity

This project brought together a diverse team of highly qualified researchers from the University of Alberta, end users from AB Distillers, and advisors from ACWA. A total of 10 highly qualified personnel (HQSPs) were trained, including three postdoctoral researchers, four PhD students, and three MSc students. The postdocs provided theoretical and hands-on training to graduate students in advanced engineering design, microbiological analysis, and ecological shift evaluation. Graduate students conducted bioreactor studies and received training in environmental biotechnology, engineering design, kinetic modeling, and molecular biology. Currently, all trainees are continuing their academic pursuits in Alberta and abroad.

With the support of this project, Dr. Liu's laboratories have made significant advancements. Several laboratory-scale and one pilot-scale reactor were procured, set up, and operated both in Dr. Liu's labs and on-site at AB Distillers' facility. These developments have strengthened research capabilities while also enhancing safety training for HQSPs, better preparing them for transitions to industry or government roles. Additionally, researchers and employees from industrial partners received training in DWW treatment, GS-based reactor operation, and process optimization, equipping them with valuable expertise to support advancements in wastewater management at a national level.

9. RECOMMENDATIONS AND NEXT STEPS

In general, a long-term plan for the commercialization of a newly developed technology involves a strategic and phased approach aimed at transitioning from research and development to practical application, widespread adoption, and commercial success and usually encompasses the following several key stages:

1. **Technology Refinement and Optimization:** The technologies generated are currently further refined and optimized based on the research outcomes. This involves addressing any identified limitations, enhancing efficiency, and fine-tuning the solution for practical use.
2. **Intellectual Property Protection:** Intellectual property rights through patents or other forms of protection will be secured for other technologies, depending on the nature of the innovation.
3. **Pilot-Scale Testing:** Before full-scale commercialization, the technologies need undergo pilot-scale testing to validate its effectiveness and real-world performance. This testing phase will provide valuable data for fine-tuning and ensures that the innovation meets industry and regulatory standards.
4. **Market Research and Analysis:** Comprehensive market research will be conducted to identify potential customers, market demand, and competitors.
5. **Business Model Development:** A robust and sustainable business model will be established, outlining revenue generation strategies, pricing structures, distribution channels, and customer acquisition plans.
6. **Funding and Investment:** Securing the necessary funding or investment to support commercialization efforts is critical. This may involve seeking venture capital, government grants, or private sector partnerships.
7. **Regulatory Approval:** Depending on the nature of the innovation, obtaining the required regulatory approvals and certifications is essential to ensure compliance with industry and environmental standards.
8. **Marketing and Sales Strategy:** A comprehensive marketing and sales strategy will be developed to promote the technology or knowledge to potential customers. This may involve targeted advertising, participation in industry events, and engagement with stakeholders.
9. **Scaling and Production:** Once the technology is proven and market demand is established, efforts will be made to scale up production or implementation to meet the needs of a broader customer base.
10. **Quality Control and Continuous Improvement:** Quality control measures will be put in place to maintain consistent product or service quality. Continuous improvement processes will also be initiated to stay competitive and adapt to evolving market conditions.
11. **Customer Support and Feedback:** Providing excellent customer support and actively seeking feedback from users is essential for building trust and improving the technology over time.
12. **Sustainability and Impact Assessment:** Continuously assessing the environmental and social impact of the technology and making necessary adjustments to enhance sustainability.
13. **Global Expansion:** If applicable, expansion into international markets may be considered, involving adaptation to local regulations and market dynamics.
14. **Long-Term Partnerships:** Establishing long-term partnerships with stakeholders, distributors, and collaborators to ensure ongoing success and market penetration.
15. **Monitoring and Adaptation:** Regular monitoring of performance metrics, customer feedback, and market trends allows for adaptability and responsiveness to changes in the business environment.

Dr. Liu's team has spent several years refining and optimizing the integrated process for distillery wastewater treatment. Through extensive research, they have demonstrated the technology's feasibility and effectiveness at the pilot-scale and collected critical data to support full-scale engineering design. Building on this foundation, the next step is to implement the process at full scale in collaboration with the industry partner. Dr. Liu and her team have proposed the integrated treatment process for full-scale demonstration to the partner, and discussions for the next development phase have been initiated.

To support project delivery, the team has established strong partnerships with an engineering company and contractors. Upon receiving approval to proceed, Dr. Liu's team will collaborate with the industrial partner to secure leveraged funding to facilitate the full-scale implementation, complete all-discipline design and construction in coordination with the engineering company, and pursue intellectual property protection. They will also engage in knowledge transfer, market assessment, and planning for long-term technology adoption. To further support commercialization, the team will seek additional investors and strategic partners to accelerate the implementation of the developed process.

10. KNOWLEDGE DISSEMINATION

10.1 Key Meetings

1) Project meeting with Alberta Innovates on February 19, 2025.
2) Project meeting with AB Distillers on November 27, 2024.
3) Project kick-off meeting with AB Distillers and Alberta Innovates on December 14, 2023.
4) Project meeting with AB Distillers on October 16, 2023.

10.2 Publications and Conferences

1) Zhang, Y., Sun, H., Huang, Q., Zhang, L., Zou, X., Liu, Y. (2025) Anaerobic digestion performance and microbial community structures in a pilot-scale up-flow anaerobic sludge blanket (UASB) treating distillery wastewater. Under review.
2) Sun, H., Huang, Q., Liu, Y. (2025) Anaerobic Digestion of Highly Alkaline Spent Caustic from a Distillery through Cultivation of Microbial Consortia. Under review.
3) Zhang, L., Yuan, Y., Li, C., Zhang, Y., Sun, H., Xu, R., Liu, Y. (2024) Biomineralization of phosphorus during anaerobic treatment of distillery wastewaters. Science of The Total Environment, 925, 171431.
4) Zhang, L., Yuan, Y., Li, C., Zhang, Y., Sun, H., Xu, R. and Liu, Y. Biomineralization of phosphorus during anaerobic treatment of distillery wastewaters. Circular Economy for Climate and Environment 1st CECE conference, 26-27 September 2023, Sydney.

11. CONCLUSIONS

This project aimed to develop and optimize a cost-effective pilot-scale process for treating high-strength distillery wastewater (DWW). Given the potential inhibitions associated with DWW, a granular sludge (GS)-based process was selected for its resilience to shock loads and strong contaminant removal performance. This goal was successfully achieved through a multidisciplinary collaboration, integrating wastewater treatment expertise, reactor engineering, and environmental biotechnology from Dr. Liu's team at the University of Alberta with operational insights from Alberta Distillers.

The project was structured in two phases: Phase I focused on developing and optimizing an anaerobic granular sludge (An-GS) process for organic removal, biomethane production, and phosphorus recovery. Phase II involved the operation of an energy-efficient aerobic/anoxic granular sludge (A/O-GS) process to further treat the An-GS effluent, achieving ammonia removal. The integrated process successfully removed over 98% of biological oxygen demand (BOD) and 99% of ammonia. Key data on process control, footprint, and waste management strategies were collected, leading to the proposal of a full-scale integrated treatment system.

The insights gained from this project provide valuable knowledge for designing more sustainable, on-site industrial wastewater treatment systems. By advancing cost-effective and environmentally responsible solutions, this work contributes to both industry sustainability and environmental protection.

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