

UNIVERSIDAD PERUANA UNIÓN
FACULTAD DE INGENIERIA Y ARQUITECTURA
Escuela Profesional de Ingeniería Ambiental



**Biomethanization of yeast, bran and sewage sludge generated by
a ethanol fermentation industry in the jungle of Peru**

Tesis para obtener el Título Profesional de Ingeniero Ambiental

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DECLARACIÓN JURADA DE ORIGINALIDAD DE TESIS

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DECLARO:

Que la presente investigación titulada: “**BIOMETANIZACIÓN DE LEVADURA, AFRECHO Y LODOS RESIDUALES GENERADOS POR UNA INDUSTRIA DE FERMENTACIÓN DE ETANOL EN LA SELVA DE PERÚ**”, de los autores **Ramiro Rufino Cruz Pinares** y **Mary Yudith Cañazaca Mamani** tiene un índice de similitud de 10% verificable en el informe del programa Turnitin, y fue realizada en la Universidad Peruana Unión bajo mi dirección.

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con el propósito de administrar el acto académico de sustentación de la tesis titulado: Biotransformación de levadura, apilado y lodos residuales generados por una industria de fermentación de etanol en la selva de Perú

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Biomethanization of yeast, bran and sewage sludge generated by a ethanol fermentation industry in the jungle of Peru

ABSTRACT

The treatment of industrial effluents with a high content of organic matter is one of the main challenges currently faced by the ethanol fermentation industry, generating different types of waste and its inadequate disposal can cause negative effects on the environment, including greenhouse gas emissions. greenhouse effect, as an agro-industrial, has the possibility of being used in energy production, through the production of biogas. This study investigates the potential for biomethanation of waste yeast supernatant (RSL), bran (RA) and sludge (RL) using anaerobic digestion technology at mesophilic temperature. The waste was collected from a beer production industry located in the Peruvian jungle. The accumulated biogas productions were 659.33 mL/g SV for RSL, 612.72 mL/g SV for RA, and 691.71 mL/g SV for RL. Regarding the concentration of methane in the biogas, the values were 58.47% for RSL, 56.56% for RA and 60.94% for RL. The results obtained in the present research demonstrate that the problem of waste in the brewing industry can be effectively converted into an environmentally friendly solution for the production of biogas using the Anaerobic Digestion process, and is also a reference for other industries similars.

Keywords: Anaerobic digestion; biogas; methane; yeast supernatant; bran; sludge.

Biometanización de levadura, afrecho y lodos residuales generados por una industria de fermentación de etanol en la selva de Perú

RESUMEN

El tratamiento de los efluentes industriales con alto contenido de materia orgánica es uno de los principales retos que afronta en la actualidad la industria de fermentación de etanol generando diferentes tipos de residuos y su disposición inadecuada puede causar efectos negativos al ambiente, incluyendo emisiones de gases de efecto invernadero, como residuo agroindustrial, tiene la posibilidad de utilización en la producción de energía, mediante la producción de biogás. Este estudio investiga el potencial de biometanización de metano de residuos de sobrenadante de levadura (RSL), afrecho (RA) y lodos (RL) usando la tecnología de digestión anaerobia a temperatura mesofílica. Los residuos fueron recolectados de una industria productora de cerveza ubicado en la selva peruana. Las producciones acumuladas de biogás fueron de 659.33 mL/g SV para RSL, 612.72 mL/g SV para RA y 691.71 mL/g SV para RL. En cuanto a la concentración de metano en el biogás, los valores fueron de 58.47% para RSL, 56.56% para RA y 60.94% para RL. Los resultados obtenidos en la presente investigación demuestran que el problema de los residuos en la industria de elaboración de cerveza puede ser eficazmente convertidos en una solución ambientalmente amigable para la producción de biogás utilizando el proceso de digestión anaerobia, y también es una referencia para otras industrias similares.

Palabras clave: Digestión anaerobia, biogás; metano, sobrenadante de levadura, afrecho y lodos.

1. Introduction

The beverage and food industries are significant sectors in many countries, with considerable impact in terms of employment. Specifically, the alcoholic beverage sector has large-scale production worldwide (de Diego-Díaz, Fernández-Rodríguez, Vitas, & Peñas, 2018). Beer is the fifth most consumed beverage globally, with an average consumption of 23 L/per year, ranking just below tea, carbonated drinks, milk, and coffee (Sangeetha, Rajneesh, & Yan, 2020). Consequently, the brewing industry generates substantial amounts of waste, primarily wastewater, spent grain, surplus hops, spent raw materials, etc. (de Diego-Díaz, Fernández-Rodríguez, Vitas, & Peñas, 2018). These wastes should be treated efficiently before discharge into the environment to prevent severe pollution problems. Due to the wide range and complexity of waste, many treatment techniques are currently used, such as composting, livestock feed, or biological processes (Jiménez, Borja, Martín, & Raposo, 2005) (Meyer & Edwards, 2014).

In general, brewery waste is easily biodegradable as the BOD/COD ratio is in the range of 0.6-0.7 (Rao, et al., 2007). Among biological treatments, anaerobic digestion (AD) is the most promising (Xu, et al., 2013). AD involves the conversion of organic matter into methane-rich biogas through a series of interconnected processes such as hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Sangeetha, Rajneesh, & Yan, 2020). Several studies have investigated the use of brewery waste for anaerobic treatment in various reactor configurations (Enitan, Adeyemo, Swalaha, & Bux, 2015) (Xu, et al., 2013). The upflow anaerobic sludge blanket (UASB) reactor is one of the most representative due to its high biomass concentration and rich microbial diversity (Xu, et al., 2013). For example, pioneering research using the UASB reactor for the treatment of brewery waste was

conducted by (Rao A. G., et al., 2007). They achieved a 96% reduction in COD and methane production of up to 0.32 m³ CH₄/g COD.

Despite the advantages offered by AD in treating industrial waste (primarily liquid waste), there is still a significant challenge in treating waste with high solids content (Sganzerla, et al., 2022), as studied in the present research. High-solids AD has received much attention recently (Rocamora, et al., 2020). (Bi, et al., 2020) evaluated the AD of chicken manure, finding a production of 0.35 m³ CH₄/kg solids, a similar production profile was found for the organic fraction of municipal waste (Di Maria, Barratta, Bianconi, Placidi, & Passeri, 2017), corn waste (Brown, Shi, & Li, 2012), among other industrial waste. Thus, due to the high organic load of waste from the brewing industry, it has great potential for bioenergy production (biogas), providing an environmentally friendly solution.

In this study, the conversion of brewery waste to bioenergy using AD technology was examined using the Biochemical Methane Potential (BMP) tests. The analyzed waste included sludge, spent grain, and lyes. The results were reported to demonstrate the biogas production potential of the company as a model solution for other industries.

2. Materials and Methods

2.1. Substrates and Inoculum

The study included three substrates generated by the ethanol fermentation industry: yeast supernatant waste (YSW), spent grain waste (SGW), and sludge waste (SW). The three wastes were collected from the effluents of a brewery located in the Peruvian jungle. Samples of 5L each were collected for the respective laboratory tests and analyses; they were stored in plastic containers, immediately frozen, and transported to the experimental

laboratories of the Environmental Engineering Department at the Universidad Peruana Unión, Juliaca campus.

The inoculum was collected from an 8 m³ biodigester operated at 23°C and fed with bovine manure. The inoculum was first sieved to remove large particles greater than 2mm, then incubated for 5 days at 32°C to minimize endogenous methane production and acclimate to the temperature at which the experiments were conducted.

2.2. Experimental Setup

Biomethanization tests were conducted using 120 ml glass bottles with a total volume of 120 ml and a working volume of 60 ml. These bottles acted as AD reactors for methane production. The experiments were conducted using the Biochemical Methane Potential (BMP) tests, following the methodology proposed by (Holliger, et al., 2016). All tests were conducted in triplicate. A substrate-free control was included to measure the endogenous methane production of the inoculum. The inoculum-to-substrate ratio (ISR) used in this study was 2 (based on VS). Immediately after feeding each bottle with the substrate and inoculum, the headspace was gassed with N₂ for 30 seconds, then the containers were sealed with butyl rubber stoppers and finally crimped with aluminum caps to maintain anaerobic conditions and prevent possible biogas leaks. The bottles were then placed in an incubator at a constant temperature of 35°C.

Methane and biogas produced during the tests were quantified using the Gas Density Biochemical Potential Test (GD-BMP) method (Justesen, et al., 2019). The methane and biogas volumes were adjusted to standard conditions, i.e., 100 kPa pressure and a temperature of 273.15 K. The GD-BMP method is mainly based on measuring the mass loss

of the bottle and the vented biogas volume at each sampling event, from which the density and composition of the biogas are obtained.

Liquid samples were taken at the beginning of the tests and also at the end of each anaerobic digestion process for respective physicochemical analyses.

2.3. Analytical Methods

Total solids (TS) and volatile solids (VS) content were determined by heating the samples in an oven (BINDER, Germany) at 105°C for 24 h and in a muffle furnace (PROTHERM) at 550°C for 1 h, respectively (APHA; AWWA; WEF, 2017). For pH, volatile fatty acids (VFA), and alkalinity (ALK) analyses, the samples were centrifuged at 5000 rpm for 30 min, and the supernatant was used for analysis. The pH value was measured using a pH meter (Horiba, Japan). VFA and ALK were quantified using the titrimetric method. All analyses were conducted at both the beginning and end of the tests.

2.4. Statistical Analysis

The obtained data were subjected to statistical analysis. Variance analysis at a 95% confidence level was performed to determine if there were significant differences between treatments. Additionally, ANOVA was applied to evaluate the significance of methane production among the three substrates. Microsoft Excel software was used. The values obtained in this research were weighted average data.

3. Results and Discussion

3.1. Waste Characterization

Table 1 summarizes the characteristics of the YSW, SGW, and SW residues. These values indicate the potential of the residues for biogas production.

Table 1. *Physicochemical characterization of YSW, SGW, and SW residues*

Parameter	Unit of Measurement	YSW	SGW	SW
TS	g/kg	205.57	252.11	345.73
VS	g/kg	172.34	225.43	50.6
pH	-	4.71	5.05	5.43
VFA	g CH ₃ COOH/L	18.15	19.84	13.32
ALK	g CaCO ₃ /L	11.2	13.73	9.81
Buffer capacity	g CH ₃ COOH/g CaCO ₃	1.62	1.45	1.36

The substrates YSW, SGW, and SW have high VS values. These values indicate the potential of the substrates in terms of biogas generation. However, factors such as pH, VFA, and ALK can alter microorganism activity and cause failures in the AD process (Maria, et al., 2023). All three substrates present acidic characteristics, with a low pH (below 5.5), high VFA concentration, and consequently low buffer capacity. According to previous studies, the optimal pH range for improving microbial growth should be between 6.8 and 8.2 (Hagos, Zong, Li, Liu, & Lu, 2017) (Franke-Whittle, Walter, Ebner, & Insam, 2014). Due to the characteristics of the substrates, it is necessary to control the operating conditions during the AD process. These aspects will be detailed in the following section.

3.1. Process Stability

Table 2 shows the results of the parameters monitored during the BMP tests to evaluate the stability of the AD process. pH, VFA, ALK, and VFA/ALK were monitored.

Table 2. Evolution of pH, VFA, ALK, and VFA/ALK at the start and end of the BMP tests

Parameter	YSW		SGW		SW	
	Start	End	Start	End	Start	End
pH	8.4	7.93	8.4	7.8	8.4	7.9
VFA (g CH ₃ COOH/L)	560	720	560	870	560	680
ALK (g CaCO ₃ /L)	1133	2275	1133	2175	1133	2100
VFA/ALK (g CH ₃ COOH/g CaCO ₃)	0.49	0.32	0.49	0.4	0.49	0.33

At the start of the experiments, the acidic pH of the substrates was balanced by the inoculum used. The pH of the inoculum-substrate mixtures at the start of the tests was 8.4, slightly above the recommended range (6.7 to 8.2) for optimal AD performance. All tests showed a slight decrease in pH at the end, which could be attributed to the hydrolysis and acidogenesis stages, where organic substrates are transformed into soluble compounds, later degraded into hydrogen, carbon dioxide, and VFA (Cucina, Pezzolla, Tacconi, & Gigliotti, 2021). Another explanation for the pH decrease is the increase in VFA concentration from 560 at the beginning to 680-870 at the end. The accumulation of VFA might be due to an imbalance between acid producers and consumers (Akuzawa, et al., 2011). However, the VFA accumulation was not significant and did not affect the microbial community; thus, methane production continued steadily.

The highest VFA concentration was observed in SGW, correlating with the lowest biogas and methane production. The VFA in SW is the lowest compared to the other

substrates, consequently yielding higher biogas and methane production. These data confirm the importance of VFA in AD process stability (Cucina, Pezzolla, Tacconi, & Gigliotti, 2021).

The VFA/ALK ratio was also investigated in the AD tests to study the effectiveness of the buffer capacity. The VFA/ALK results at the end of the tests (0.32, 0.4, and 0.33 g CH₃COOH/g CaCO₃ in YSW, SGW, and SW, respectively) show an improvement compared to the beginning (0.49 g CH₃COOH/g CaCO₃). The optimal range for a stable AD process is between 0.2 – 0.6 (Di Maria, et al., 2014) (Tacconi, Cucina, Pezzolla, Zadra, & Gigliotti, 2018). All experiments showed optimal VFA/ALK values, demonstrating a good balance between the acidogenic and methanogenic stages (Cucina, Pezzolla, Tacconi, & Gigliotti, 2021).

The pH, VFA, and VFA/ALK during the AD process in all experiments suggest that the substrates (YSW, SGW, and SW) can produce good amounts of biogas without risks of inhibition.

3.2. Biogas Production and Composition

To estimate the biogas and methane content of residues from a brewery, BMP tests were conducted for approximately 56 days using YSW, SGW, and SW, as shown in Figures 1 and 2. Similar trends in biogas production were observed in all three substrates. Biogas production starts immediately from day 0, with high, constant production until approximately day 30. From day 31 to 56, biogas production is lower, with maximum biogas production reached around day 30.

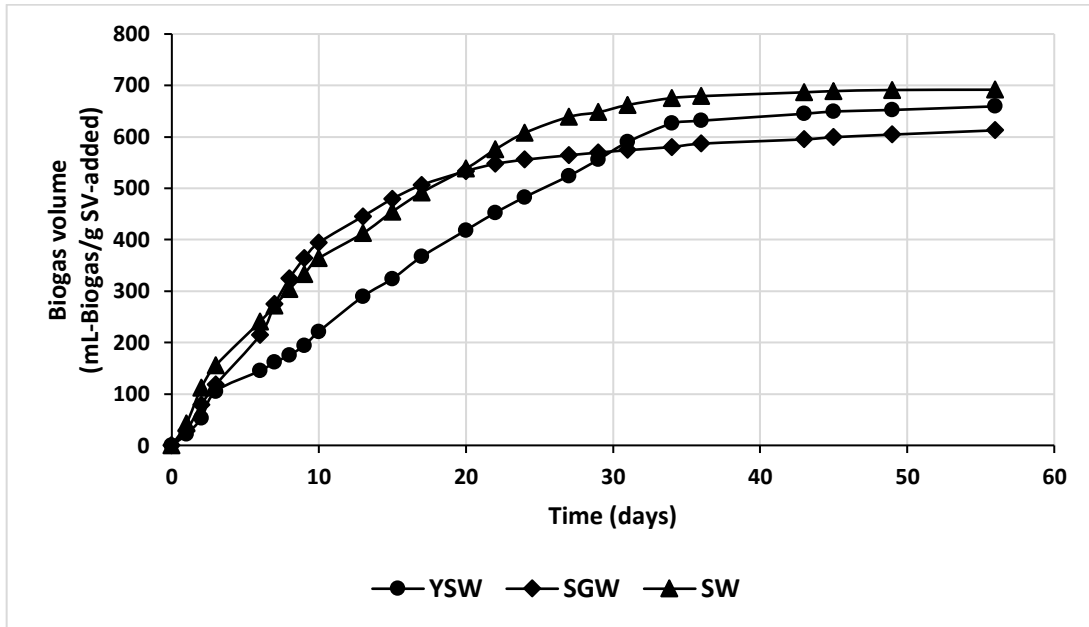


Figura 1. *Cumulative Biogas Production with the substrates: YSW, SGW, and SW.*

Biogas production decreases in the order $SW > YSW > SGW$, with values of 691.71, 659.33, and 612.72 mL/gVS, respectively. These results are much higher than other residues. (de Diego-Díaz, Fernández-Rodríguez, Vitas, & Peñas, 2018) evaluated the biomethanization of barley malt and sloe berries from the alcoholic beverage industry at two temperatures (35°C and 55°C) with thermal pretreatment (80°C for 1.5h), obtaining biogas productions in the range of 95 to 319 mL/gVS.

It was also observed that the organic matter content slightly affects total biogas production; that is, the higher the organic matter (VS), the lower the biogas production (Caliskan, Giray, gundogdu, & Azbar, 2014). For example, the biogas production of SW (VS of 50.6 g/kg) is higher than YSW (VS of 172.34 g/kg) and SGW (VS of 225.43 g/kg). All the biogas production results were high because the AD process developed stably, as pH, VFA, ALK, and VFA/ALK values were within the permissible range, as shown in the previous section.

Regarding biogas composition (Figure 2), it remains constant throughout the AD process. After day 10 of operation, all reactors reached stability, remaining between 50 to 72%. A slight drop can be observed from day 1 to 7, with a minimum concentration of around 32% for YSW. On average, the methane content of YSW, SGW, and SW was 58.47, 56.56, and 60.94%, respectively. These results confirm the importance of not overloading the reactors with too much organic matter, as microbial activity may decrease due to increased organic matter content (Sganzerla, et al., 2022).

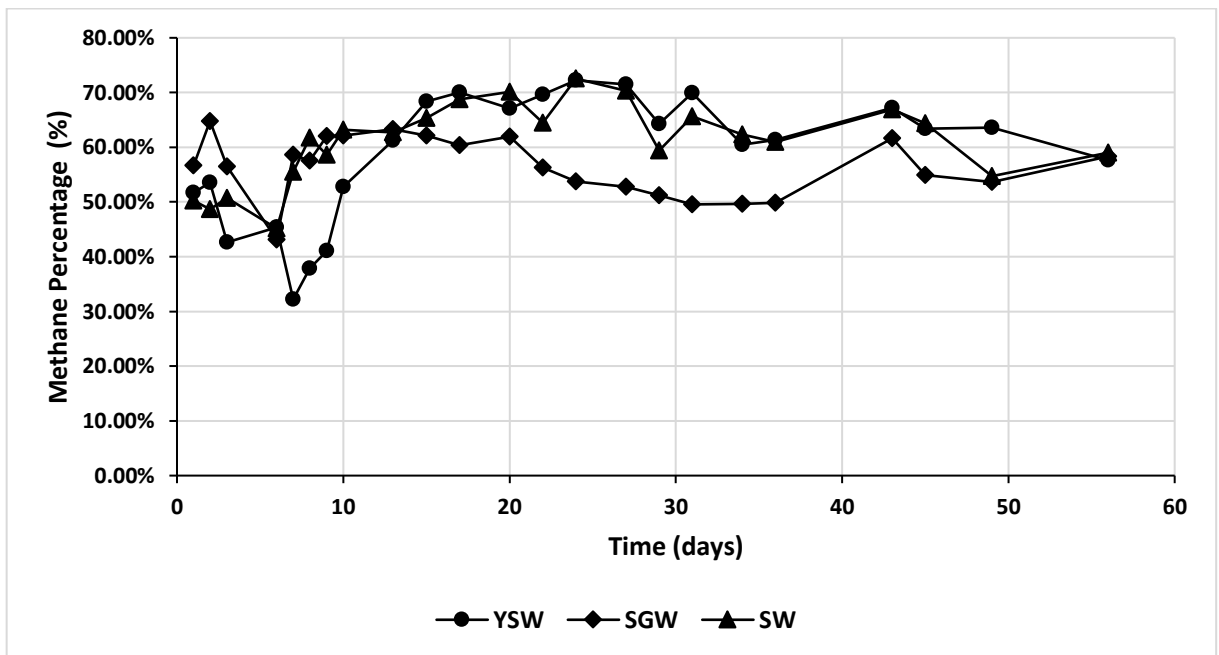


Figura 2. Methane Concentration in Biogas (% of methane) with the substrates: Yeast Supernatant, Spent Grain, and Sludge.

All results obtained, from characterization to biogas composition, of the AD process of YSW, SGW, and SW show the potential and efficiency in biogas and methane production compared to other reported results using beer lees (Sun, et al., 2019), brewery wastewater (Caliskan, Giray, gundogdu, & Azbar, 2014), and opaque beer wastewater (Manyuchi, Mbohwa, & Muzenda, 2018). Furthermore, biogas production is higher than other types of

residues, such as chicken manure (Bayrakdar, Sürmeli, & Calli, 2017), food waste (Nguyen, et al., 2017), and municipal solid waste (Ni, Liu, & Zhang, 2017). Table 3 summarizes the main values obtained in this research.

Table 3. Summary of BMP test results for yeast supernatant, spent grain, and sludge

Parameter	Unit of Measurement	Yeast Supernatant	Spent Grain	Sludge
Biogas Production	mL - Biogas/g VS - added	659.33	612.72	691.71
Methane Production	mL-CH ₄ /g VS- added	389.15	343.24	408.35
Methane Concentration in Biogas	%	58.47	56.56	60.94
Time	Days	56	56	56

4. Conclusions

Waste management in the alcoholic beverage production industry is a critical issue. YSW, SGW, and SW residues are highly biodegradable and can be efficiently treated using AD technology due to the high biogas productions (612.72 – 691.71 mL / gVS) rich in methane (56.56 – 60.94%). The methanogenic reactions were stable due to the pH, VFA, ALK, and VFA/ALK values during AD.

5. Acknowledgement

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7. ANEXOS

7.1 Evidencia de Sumisión del artículo

The screenshot shows the 'Author Dashboard / Submission Confirmation' page. At the top, it displays 'ScholarOne Manuscripts' and the date '09/08/24, 10:58 a. m.'. The journal logo 'SciELO Revista Ambiente & Agua' is visible. The page title is 'Submission Confirmation', followed by the message 'Thank you for your submission'. The submission details are as follows:

Submitted to	Revista Ambiente & Agua
Manuscript ID	AMBIAGUA-2024-3028
Title	Biomethanization of yeast, bran and sewage sludge generated by a ethanol fermentation industry in the jungle of Peru
Authors	Cruz, Ramiro Madrigal Pérez, Glen Bryan Cañazaca, Mary Vigo, Juan
Date Submitted	09-Aug-2024

A 'Author Da' button is located in the bottom right corner of the page.

7.2 Resolución de inscripción del perfil del Proyecto de Tesis en formato artículo



"AÑO DEL BICENTENARIO, DE LA CONSOLIDACIÓN DE NUESTRA INDEPENDENCIA, Y DE LA CONMEMORACIÓN DE LAS HEROICAS BATALLAS DE JUNÍN Y AYACUCHO"

RESOLUCIÓN N° 0047-2024/UPeU-FIA-CF-T

Lima, Ñaña 06 de febrero de 2024

VISTO:

El expediente de **Mary Yudith Cañazaca Mamani**, identificado(a) con código universitario N° 201521957 y **Ramiro Rufino Cruz Pinares** identificado(a) con código universitario N° 201410575 de la Escuela Profesional de Ingeniería Ambiental de la Facultad de Ingeniería y Arquitectura de la Universidad Peruana Unión;

CONSIDERANDO:

Que la Universidad Peruana Unión tiene autonomía académica, administrativa y normativa, dentro del ámbito establecido por la Ley Universitaria N° 30220 y el Estatuto de la Universidad;

Que la Facultad de Ingeniería y Arquitectura de la Universidad Peruana Unión, mediante sus reglamentos académicos y administrativos, ha establecido las formas y procedimientos para la aprobación e inscripción del proyecto de tesis;

Que **Mary Yudith Cañazaca Mamani** y **Ramiro Rufino Cruz Pinares**, han solicitado la modificación de la denominación del proyecto de tesis titulado "Efecto de *Suillus luteus* en la producción de biogás a partir de estiércol vacuno en condiciones Altoandinas";

Estando a lo acordado en la sesión del Consejo de la Facultad de Ingeniería y Arquitectura de la Universidad Peruana Unión, celebrada el 06 de febrero de 2024, y en aplicación del Estatuto y el Reglamento General de Investigación de la Universidad;

SE RESUELVE:

Aprobar la modificación de la denominación del proyecto de tesis titulado "Efecto de *Suillus luteus* en la producción de biogás a partir de estiércol vacuno en condiciones Altoandinas", por el de: "Biometanización de levadura, afrecho y lodos residuales generados por una industria de fermentación de etanol en la selva de Perú", en el registro respectivo y disponer que con la orientación de su asesor el(la) **Dr. Jael Calla Calla**, sea desarrollado y ejecutado el proyecto de tesis por **Mary Yudith Cañazaca Mamani** y **Ramiro Rufino Cruz Pinares**, otorgándoles un plazo máximo de doce (12) meses para la ejecución, a partir de la inscripción inicial.

Regístrese, comuníquese y archívese.




Dra. Erika Inés Acuña Salinas
DECANA




Mg. Ketty Magaly Arellano Lino
SECRETARIA ACADÉMICA

CC:

- Interesado
- Asesor
- DGI
- Archivo

7.3 Resolución de sustentación de Tesis



“AÑO DEL BICENTENARIO, DE LA CONSOLIDACIÓN DE NUESTRA INDEPENDENCIA, Y DE LA CONMEMORACIÓN DE LAS HEROICAS BATALLAS DE JUNÍN Y AYACUCHO”

RESOLUCIÓN N° 0619-2024/UPeU-FIA-CF

Lima, Naña, 13 de agosto de 2024

VISTO:

El expediente de los (las) bachilleres **Mary Yudith Cañazaca Mamani** identificado(a) con código universitario N° 201521957 y **Ramiro Rufino Cruz Pinares** identificado(a) con código universitario N° 201410575, de la Escuela Profesional de Ingeniería Ambiental de la Facultad de Ingeniería y Arquitectura de la Universidad Peruana Unión;

CONSIDERANDO:

Que la Universidad Peruana Unión tiene autonomía académica, administrativa y normativa, dentro del ámbito establecido por la Ley Universitaria N° 30220 y el Estatuto de la Universidad;

Que la Facultad de Ingeniería y Arquitectura de la Universidad Peruana Unión, mediante sus reglamentos académicos y administrativos, ha establecido las formas y procedimientos para la sustentación de la tesis en formato artículo;

Que el Comité Dictaminador ha emitido su dictamen aprobando el informe de tesis titulado "Biometanización de levadura, afrecho y lodos residuales generados por una industria de fermentación de etanol en la selva de Perú", presentado por los(las) bachilleres **Mary Yudith Cañazaca Mamani** y **Ramiro Rufino Cruz Pinares**, reuniendo de esta manera las condiciones previas para la declaratoria de expedito para la programación de la sustentación;

Estando a lo acordado en la sesión del Consejo de la Facultad de Ingeniería y Arquitectura de la Universidad Peruana Unión, celebrada el 13 de agosto de 2024, y en aplicación del Estatuto y el Reglamento General de Investigación de la Universidad;

SE RESUELVE:

1. Declarar expedito a los (las) bachilleres **Mary Yudith Cañazaca Mamani** y **Ramiro Rufino Cruz Pinares**, para que sustenten la tesis en formato artículo titulada "Biometanización de levadura, afrecho y lodos residuales generados por una industria de fermentación de etanol en la selva de Perú", conducente a la obtención del título profesional de Ingeniero Ambiental, el 29 de agosto de 2024, a las 12:00 horas, en el Auditorio Wellesley Muir.
2. Designar el Jurado de Sustentación, encargado de gestionar la sustentación respectiva, el mismo que queda constituido por los siguientes miembros:

Presidente: Ing. Enrique Mamani Cuela
Secretario: Ing. Nancy Curasi Rafael
Asesor: MSc. Jael Calla Calla
Vocal 1: MSc. Loayda Abigail Condori Turpo
Vocal 2: Mtro. Juan Eduardo Vigo Rivera

Regístrese, comuníquese y archívese.




Dra. Erika Inés Acuña Salinas
DECANA




Ph.D. Silvia Pilco Quesada
SECRETARIA ACADÉMICA

cc:
-Interesado
-Jurado (04)
-Secretaría General
-Archivo

7.4 Constancia de Inglés

CONSTANCIA

Yo, **JAEL CALLA CALLA**, docente de la E.P. Ingeniería Ambiental y especialista en redacción técnica e idioma extranjero (Inglés).

Realicé la revisión del idioma inglés del **artículo** del trabajo de investigación titulado "*Biomethanization of yeast, bran and sewage sludge generated by a ethanol fermentation industry in the jungle of Peru*" de los estudiantes: Ramiro Rufino Cruz Pinares, Mary Yudith Cañazaca Mamani, de la E.P. Ingeniería Ambiental.

Siendo el resultado el siguiente:

- Aprobado sin observaciones

Constancia que se expide a petición de la parte interesada el 11 de octubre del 2024.



Firma

CIP:146540

DNI:10250002