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優勝作品專輯

作品編號　**200006**

參展科別　環境工程

作品名稱　以酵母提升低溫環境下厭氧固定生物系統

Improvement of low-temperature anaerobic immobilized bioreactor via co-feeding yeast

就讀學校　臺北市私立復興實驗高級中學

指導教師　陳虹瑤、姚曉輝

作者姓名　荷予恩、邱思瑜

關鍵詞　**Anaerobic Reaction、Psychrophilic Temperature、Yeast**

作者簡介

我們是邱思瑜和荷予恩，目前就讀台北市私立復興高級實驗中學二年級。自高二起，很榮幸地進入林正芳教授的實驗室進行研究。這一路上真的一起經歷了太多，但也真的是一次很寶貴的經驗！同時很謝謝實驗室的虹瑤姐這一路上對我們的指導、支持、和陪伴，以及家長們的支持，常常半夜2、3點的接送…

希望透過這次的國際科展能與更多有共同興趣的夥伴們交流！

摘要

厭氧生物反應較好氧處理系統，操作成本低、耗能低、少污泥，亦生成甲烷產能，可將污水轉換為能源生成。然而常溫菌(30-40°C)代謝活性陡降於 15°C，也降低了 COD 去除率。因此，厭氧生物系統被應用於長年高溫的熱帶及亞熱帶地區，而非較高緯度地區。厭氧生物反應限制於溫度<15°C 的環境如何維持 COD 去除率，進而導致厭氧系統難以全球廣泛應用。本研究採用額外添加的兩種酵母(*Saccharomyces cerevisi* 和 *Saccharomyces pastorianus*)，觀察在低溫下可否有效提升厭氧系統溫度。而添加的酵母亦可藉由增加 COD 去除率，提升氣體產物 CO_2、CH_4 之產量。

在本研究中，於低溫控制厭氧生物系統添加酵母試驗 (1)反應槽系統溫度平均增加 4.22°C (2) COD 去除率增加 9.99%，氣相 CH_4 增加 2.9%，CO_2 增加 9.7%。於常溫操作添加酵母試驗 (3)氣相 CH_4 增加 8.7%，CO_2 增加 6.2%，(4) COD 去除率則增加 3%。研究結果驗證於低溫及常溫環境，酵母發酵可有效促進酸化反應，進一步影響甲烷生成。而添加酵母於厭氧生物系統的操作，可有效提升 COD 去除率及甲烷含量。添加酵母於厭氧生物系統之操作有低成本、生成甲烷產能的優點，將可提升在稍高緯度國家的使用優勢。

Abstract

Anaerobic biological treatment processes (ABTPs), compared to aerobic biological treatment processes, include lower operational costs and energy demands, less sludge production, and the creation of methane, a potential source of energy, as biogas throughout wastewater treatment. However, mesophilic microbes' optimal under 30 to 40°C has a drastic decrease in metabolic activity after reaching 15°C, therefore decreasing the removal rate of chemical oxygen demand (COD). As a result, ABTPs are attractive particularly to subtropical and tropical regions where the climate is consistently warm throughout the year, rather than countries of higher altitudes. Therefore, the technology of maintaining COD removal efficiency below 15°C is unmet, leading to an inability of widespread ABTP application globally. This study employed the introduction of two strains of yeast, *Saccharomyces cerevisi* and *Saccharomyces pastorianus*, as to observe whether the addition of yeast will help raise the overall temperature of the anaerobic immobilized bioreactor, contribute to gas composition by raising CO_2 and CH_4 levels, and in correspondence, increase COD removal rate. Results show that after the addition of yeast, there was (1) an average 4.22°C increase in temperature under

psychrophilic temperatures (2) an 9.99% increase in average COD removal rate, a 2.9% increase in CH_4, and a 9.7% increase in CO_2 under psychrophilic temperatures. (3) There was a 6.2% increase in CO_2 and an 8.7% increase in CH_4 of biogas (4) and a 3% increase in COD removal efficiency under mesophilic temperatures.

It can be suggested that under both mesophilic and psychrophilic conditions, the fermentation of yeast is successfully used to facilitate the process of acidogenesis, which leads to facilitation of methanogenesis. This study investigates the potential beneficial anaerobic biological interactions that help initiate a low cost and low energy demanding solution for increasing COD removal rates and increasing methane content in countries of a higher latitude, suggesting the potential introduction of yeast strains within ABTP usage in these areas.

壹、前言

一、研究動機

Aerobic wastewater treatment is globally prevalent in wastewater streams with relatively low COD levels, yet, due to the requirement of supplemental oxygen through the incorporation of aeration systems, it is not energy efficient. On the other hand, anaerobic wastewater treatment systems do not require the excess energy demands, and generates methane, in opposition to the carbon dioxide produced by Aerobic Wastewater treatment systems, which, as a hydrocarbon, yields significantly higher energy potential. Yet, issues such as the biochemical reaction kinetics of temperature have limited the widespread use of anaerobic wastewater treatments in low strength wastewater systems. A relatively low cost solution to the heating of low strength wastewater in high altitude countries to improve anaerobic reactions would be the massive generation of heat by newly introduced microorganisms. Microorganisms present a great opportunity for energy science, and hence are a natural focus for the Department of Energy. (Jason, 2016) In our study, we took advantage of the fermentation of both mesophilic and psychrophilic yeast, that is, *Saccharomyces cerevisiae* and *Saccharomyces pastorianus*, in hopes to see that metabolic activity of yeast at psychrophilic temperatures directly in the reactor would aid in raising ABR to a temperature under psychrophilic temperatures to conditions suitable for anaerobic reactions to occur, and further inducing the production of methane gas at low temperatures and maintaining a high and stable COD removal rate.

二、研究目的

(一) To observe whether the addition of yeast will help raise the overall temperature of the anaerobic immobilized bioreactor.

(二) To observe whether the addition of yeast will contribute to gas composition by raising CO_2 and CH_4 levels, and in correspondence, increase COD removal rate.

三、Abbreviations

(一) Chemical Oxygen Demand – COD, a measure of water and wastewater quality by means of the total amount of oxygen needed to fully oxidize all organic compounds into CO_2.

(二) Anaerobic Baffled Reactor – ABR, the anaerobic reaction tank used in this study that contains bioplates with implanted wastewater treatment microbial communities.

(三) Hydraulic Retention Time – HRT, the period of time that the influent stays in the ABR before coming out as effluent, calculated as shown in equation 1.

$$HRT = V \text{ (Volume of reactor)}/v \text{ (velocity of flow)} \qquad \text{(equation 1.)}$$

(四) Gas Chromatography-Thermal Conductivity Detector - GC-TCD, the machine used in this study to test gas composition.

貳、研究方法與過程

一、Experiment Flow Chart

The experiment focused on experimental groups dependent on temperature and yeast variables which were then analyzed through both the liquid and gas phase (Figure 1).

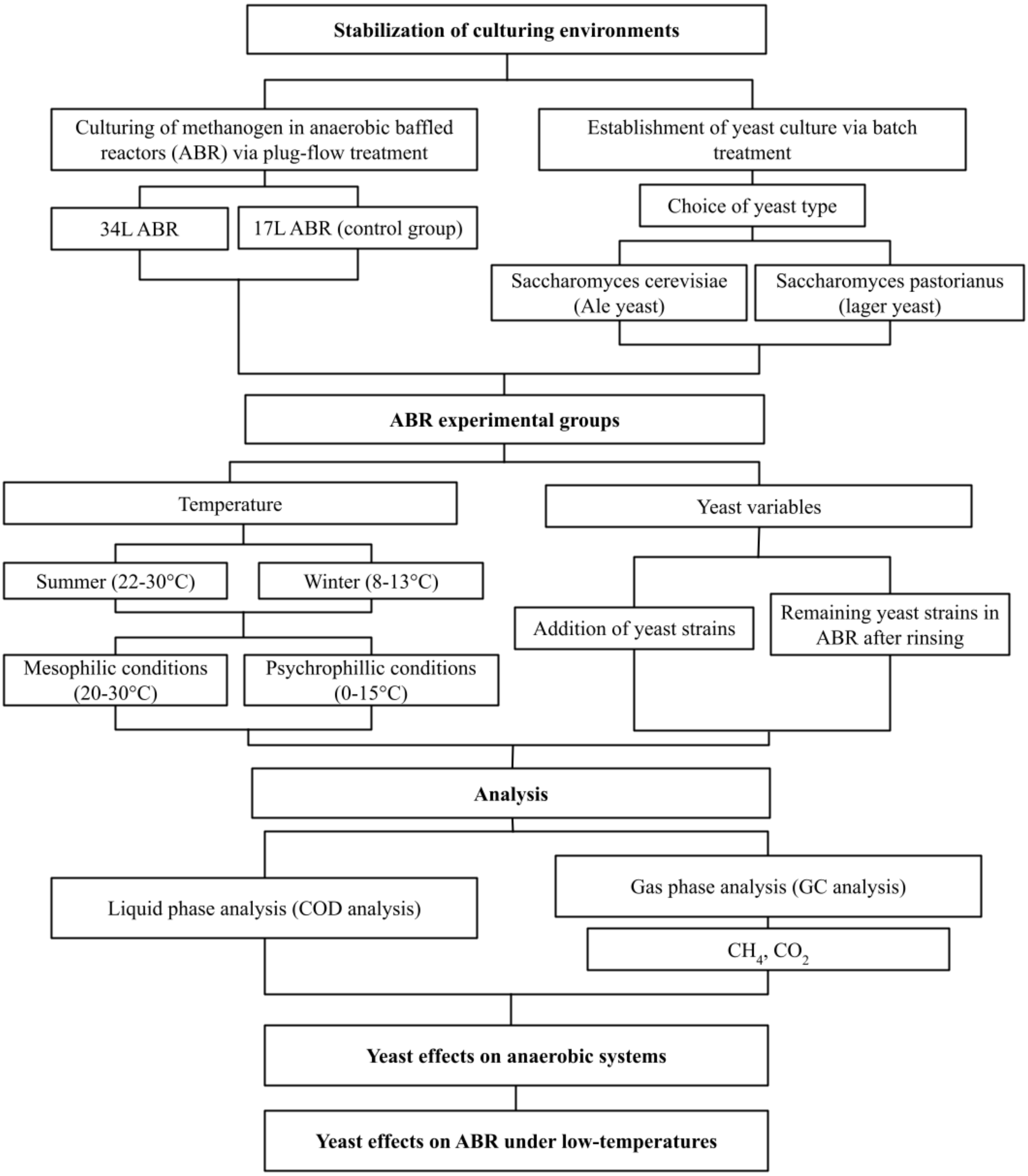

Figure 1. Experimental Flow Chart

二、Experimental Materials

(一) Anaerobic Biological Reactor (ABR)

The ABR inner tank has a volume of 24 L and the fluid level in the tank is 35 cm, with 5 cm reserved at the top for gas collection. In the tank, seven fixed biological plates are arranged by height, where the tall bioplates are followed by the short bioplates, continuing on in that consecutive order. The tall bioplates do not exceed the fluid level, and the short bioplates are uniformly parallel, with a 5 cm spacing to the bottom of the tank. The 5 cm spacings that the tall bioplates have with the fluid level and short bioplates with the bottom of the tank allows the circulation of fluids. Methanogens are fixed onto the seven biological plates, and together, the bioplates create four compartments which in total, have an effective reaction volume of 17 L. The experimental setup is shown below (Figure 2):

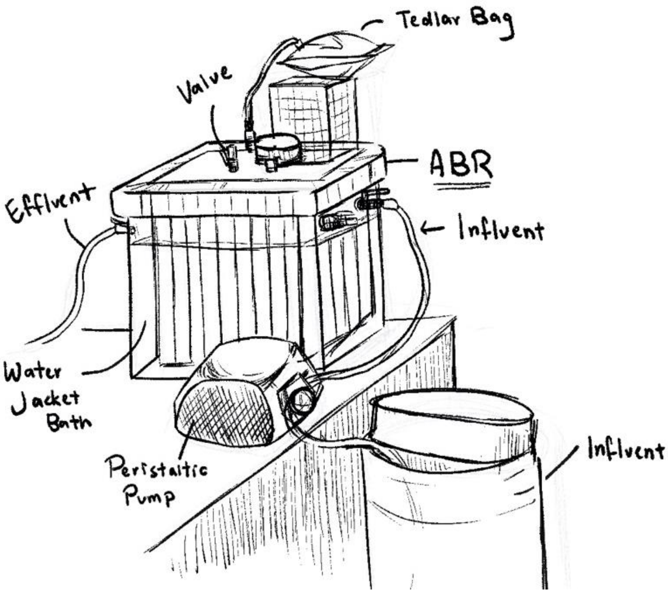

Figure 2. Set up of Anaerobic Biological Reactor – Gas Collection, Influent and Effluent, Water Jacket Bath

(二) Yeast Tank

The yeast tank is made of an airtight double-layer acrylic material containing a circular inner tank with a volume of 5 L.

(三) Water Jacket Bath

There is a 2 cm spacing between the outer and inner tank of the ABRs and the yeast tank. An external pump is connected to the water bath (Figure 3), circulating water flow of a certain temperature. This ensures the tanks' ability to stably mimic an environment at a certain temperature range.

Figure 3. Low-temperature water bath (Model: FIRSTEK B403)

(四) Inflow System

The inflow of the ABR tank is controlled by a peristaltic pump (Model: Cole Parmer 7553 Masterflex Controller and Pump), introducing the synthetic sewage into the ABR tank from the inlet bucket, while ensuring the amount of synthetic sewage in the inlet bucket at a sufficient level to prevent air from being pumped into the system.

(五) Spectrophotometer

The spectrophotometer (Model: HACH DR 3900) was used to analyze the chemical oxygen demand (COD) of the influent and effluent from the ABR tanks. There are two measurable ranges of COD concentrations, concentrations ranging from 3 to 150 mg/L COD (LR) and from 20 to 1500 mg/L COD (HR).

(六) Gas Analysis

Three gases, methane, carbon dioxide, and nitrogen, are analyzed using a gas chromatography-thermal conductivity detector. (Model: Shimadzu 2010 Plus Gas Chromatography-Thermal Conductivity Detector, GC 2010 Plus-TCD).

(七) Syringe Filters

This experiment tested only the soluble chemical oxygen demand (SCOD) for both influent and effluent solutions. Syringe filters were used to filter and collect the SCOD from the total chemical oxygen demand (TCOD) before being added into the HACH COD digestion reagent.

三、Experimental Procedures

(一) Stabilization of ABR Environments

In this experiment, two ABRs, with the volume of 34 L and 17 L, were used as the experimental group and the control group. To ensure that the second ABR can be used as the control group, a stabilization period was enacted to confirm that the experimental group ABR and the control group ABR's COD removal rates were comparable. The stabilization period lasted for one month, with both reaction tanks being continuously fed the carbohydrate-based wastewater solution listed in Table 1. via plug-flow treatment, under a hydraulic retention time (HRT) of 12 hours. Both of the tanks' influent COD, effluent COD, and produced gas were collected and tested everyday to see whether the COD removal rate and the gas composition were comparable.

Table 1. Carbohydrate-based wastewater influent model formula

Substrate	Concentration (mg/L)
sucrose	900
NH_4Cl	162
K_2HPO_4	42.2
$NaHCO_3$	600
$CaCl_2$	14.6
$FeCl_3$	13.5
$MgCl_2 \cdot 6H_2O$	5.00

(二) Establishment of Yeast Culture

Two types of yeast, *Saccharomyces cerevisiae* and *Saccharomyces pastorianus*, were individually cultured in the yeast tank for a time of 20 days before being added to the influent solution of the big ABR. For each type of yeast, 11 grams of yeast were added into 5 L of water in the yeast tank. The yeast were cultured via batch treatment whereas 14.4 g of sucrose were added every 2 days and were continuously stirred by the magnetic stirrer placed underneath. The surrounding water jacket bath of the tank was controlled at 15°C in hopes for the yeast to adapt to lower temperatures during culture. However, after the culturing of ale yeast, the water jacket bath started malfunctioning and the culturing of lager could only be kept under a stable environment of 18°C.

(三) Establishment of Mesophilic Environment

At first, a water jacket bath surrounding the ABR was set up to maintain a stable mesophilic environment. However, it was observed that the temperature the water jacket bath indicated did not match up with the actual temperature of circulating water - the temperature indicated was always slightly lower than the actual water temperature. This, however, did not influence the maintenance of a mesophilic environment, since the room temperature itself was already mesophilic. Furthermore, the water-jacket bath acted as a buffer layer, insulating the ABR tank from minor temperature changes, overall establishing a stable and controlled mesophilic environment.

(四) Establishment of Psychrophilic Environment

With the acknowledgment of the incapability of the water jacket bath to accurately set temperatures, another approach was used to mimic a psychrophilic environment. The influent temperature was set to 10°C through the replacement of mesophilic-temperature water with ice water that was scaled to the formula ratio. A plastic bag filled with ice cubes was added into the influent tank to maintain the psychrophilic temperature. Fluctuations of temperature were prevented as the ABR, water jacket bath, and influent temperatures were measured regularly every hour. If the temperatures were to rise, more ice cubes would be added into the system.

參、 研究結果與討論

To observe whether the addition of yeast will contribute to gas composition by raising CO_2 and CH_4 levels, and in correspondence, increase COD removal rate.

一、Establishment and stabilization of ABR environment in different stages with varying yeast variables

In Stage I, the ABR underwent a stabilization period, where the microbial community was simply fed the carbohydrate-based wastewater solution in Table 1. The stabilization period aimed to ensure that that the ABR microbial community could adapt to the controlled environmental factors (as shown in Table 3), therefore establishing a steady effluent COD concentration and gas production.

The controlled environmental factors included the influent concentration of the carbo-hydrate based wastewater solution, which was maintained at a COD concentration of 900 mg/L as a continuation of Tien's experiment (Tien, 2016), which controlled the carbo-hydrate based wastewater solution at a COD level between 200 mg/L and 800mg/L, and received stable effluent COD concentrations with no fluctuation. The room temperature ranged from 22°C to 30°C throughout Stages I-V, as the experiment was conducted during summer seasons.

During Stage II, *S. cerevisiae* was added into the ABR via co-feeding. Co-feeding served to subtly introduce *S. cerevisiae* to the original ABR environment, allowing suitable *S. cerevisiae* to either stay on the bio plates, or leave the ABR along with the effluent. A psychrophilic experiment was conducted at the latter stages of Stage II'.

Stage III utilized the method of rinsing to decrease the population of *S. cerevisiae* in the ABR. During rinsing, the influent was simply the carbo-hydrate based wastewater solution with no addition of *S. cerevisiae.* Through rinsing, it was anticipated that *S. cerevisiae* should have left the ABR along with the effluent. However, from COD removal rate results, it seemed that remnants of *S. cerevisiae* that had adapted to the ABR environment and become a permanent addition to the original microbial community. A psychrophilic experiment was conducted at the latter stages of Stage III when the possibility of remnants of *S. cerevisiae* being established in the ABR had been observed.

In Stage IV, the same method of co-feeding was utilized to introduce *S. pastorianus* into the ABR environment. The environmental conditions remained consistent to the Stage I, as shown in Table 2. A psychrophilic experiment was conducted at the latter stages of Stage IV when the ABR system had reached stabilization.

Stage V undergoes the rinsing method on *S. pastorianus*, to decrease the population of *S. pastorianus* in the ABR.

Stages I'-IV' followed the exact same conditions as Stages I-IV. However, the Stages I'-IV' were reconducted during winter seasons, where the room temperature ranged from 8°C to 13°C. Psychrophilic samples were taken at the latter stages of Stage II', the introduction of *S. cerevisiae*, Stage IV', the introduction of *S. pastorianus,* and Stage V', the rinsing of *S. pastorianus.* Figure 4 presents liquid phase results for every stage

Table 2. Control Variables of Various Stages

Stage	HRT	Temperature	COD_{inf} (mg/L)	Yeast Variables	Remarks
I	12	22°C-30°C	850-1200	-	-
I'	12	8°C -13°C	850-1200	-	-
II	12	22°C-30°C	850-1200	+ *S. cerevisae*	-
II'	12	8°C -13°C	850-1200	+ *S. cerevisae*	+ Psychrophilic sample
III	12	22°C-30°C	850-1200	- *S. cerevisae*	+ Psychrophilic sample
III'	12	8°C -13°C	850-1200	- *S. cerevisae*	+ Psychrophilic sample
IV	12	22°C-30°C	850-1200	+ *S. pastorianus*	+ Psychrophilic sample
IV'	12	8°C -13°C	850-1200	+ *S. pastorianus*	+ Psychrophilic sample
V	12	22°C-30°C	850-1200	- *S. pastorianus*	-
V'	12	8°C -13°C	850-1200	- *S. pastorianus*	+ Psychrophilic sample

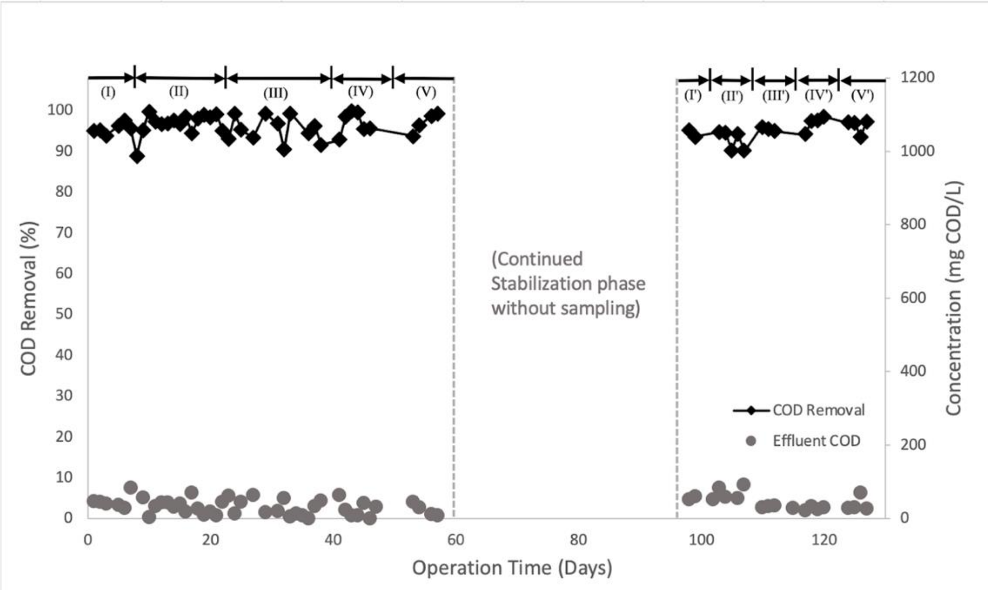

Figure 4. COD removal rate and corresponding effluent COD using ABR over extended periods with varying yeast variables

(Stage I: Stabilization of ABR, Stage II: Addition of *S. cerevisiae*, Stage III: Rinsing of *S. cerevisiae*, Stage IV: Addition of *S. pastorianus*, Stage V: Rinsing of *S. pastorianus*, Stage I': Stabilization of ABR, Stage II': Addition of *S. cerevisiae*, Stage III': Rinsing of *S. cerevisiae*, Stage IV': Addition of *S. pastorianus*, Stage V': Rinsing of *S. pastorianus*)

二、The effects of the addition of *Saccharomyces cerevisiae* and *Saccharomyces pastorianus* into ABR environment

(一) Fermentation of *Saccharomyces cerevisiae* and *Saccharomyces pastorianus*

The fermentation of yeast (including *S. cerevisiae* and *S. pastorianus*) can facilitate two stages of anaerobic reaction, acidogenesis and acetogenesis (Figure 5).

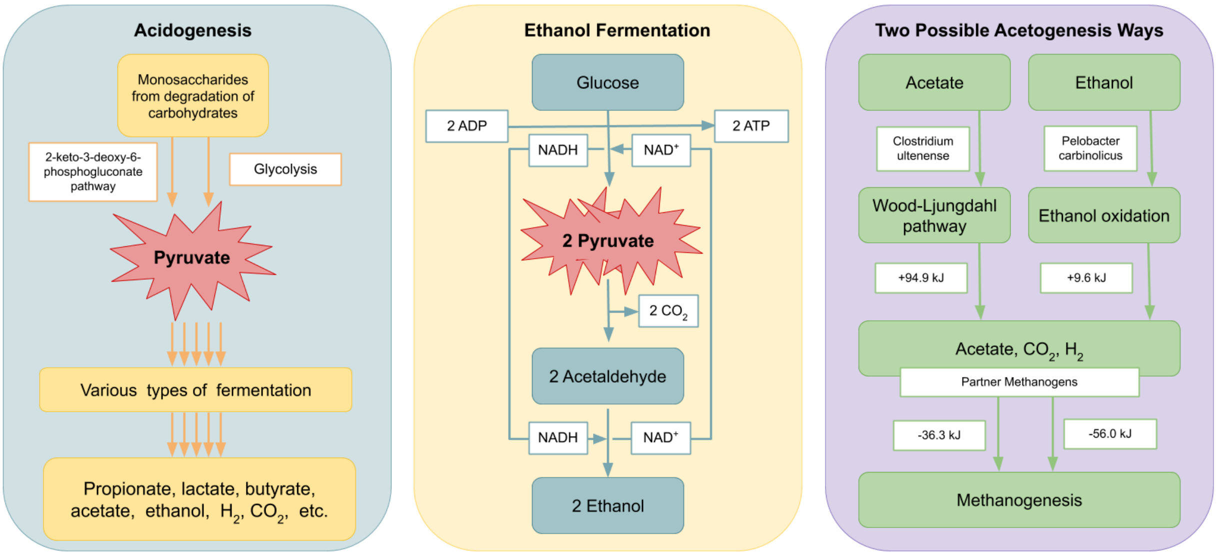

Figure 5 Comparison between acidogenesis, acetogenesis, and fermentation pathways.

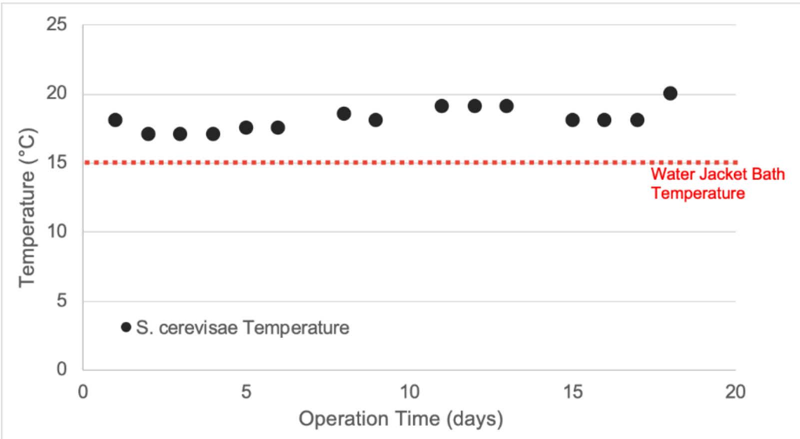

Figure 6. Rise of temperature during fermentation of *Saccharomyces cerevisiae*

Figure 6. shows the temperature increase during the fermentation of *S. cerevisiae* at a controlled environment temperature of 15°C. The optimal temperature for *S. cerevisiae* fermentation reigns from 18°C to 23°C, and in that range, fermentation should, at maximum, increase the temperature around 4°C. The average rise in temperature was 3°C under 15°C, suggesting that metabolic activity was maintained below the optimal temperature range for fermentation.

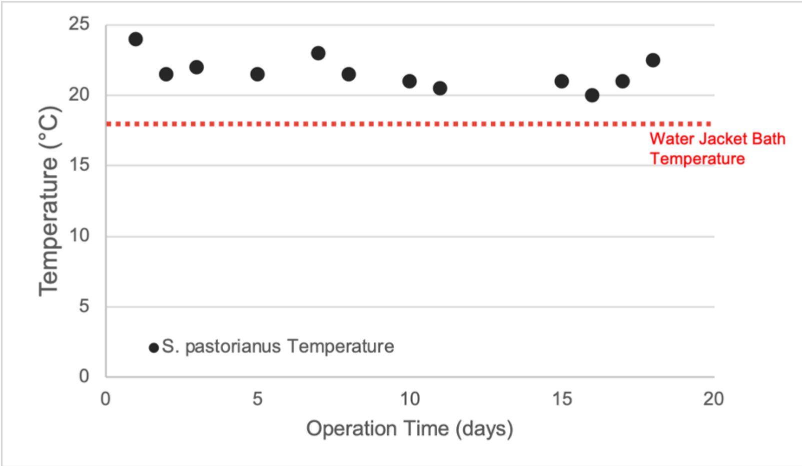

Figure 7. Rise of temperature during fermentation of *Saccharomyces pastorianus*

Figure 7. shows the temperature increase during fermentation of *S. pastorianus* at a controlled environment temperature of 18°C. The optimal temperature for *S. pastorianus* fermentation reigns from 8°C to 14°C, and in that range, fermentation should increase the temperature. The average rise in temperature was 6.5°C under 18°C, suggesting that metabolic activity was maintained at a psychrophilic temperature.

(二) Addition of *Saccharomyces cerevisiae* and *Saccharomyces pastorianus* into ABR environment at high temperature

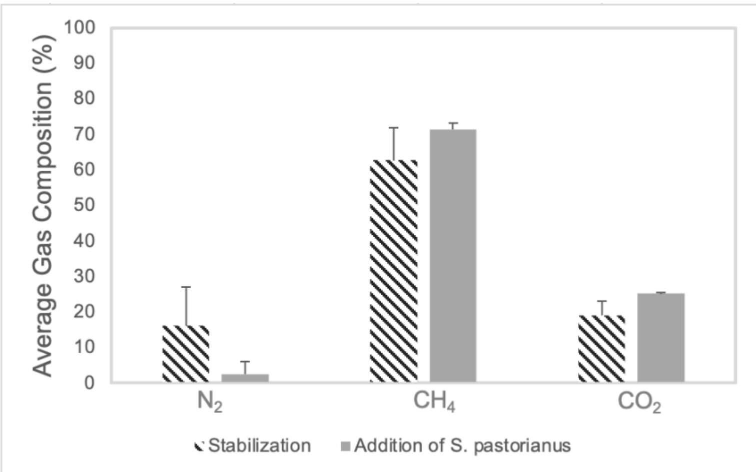

Figure 8. Average Gas makeup in Stage I and Stage IV There is a CH_4 and CO_2 increase and N_2 decrease with the addition of *S. pastorianus*.

1. Stabilization period (Stage I)

(1) In Stage I, the average COD removal rate was 95.70 % ± 1.4%.

(2) In Stage I, the average gas makeup was N_2 – 16.2% ± 10.93%, CH_4 – 62.73% ± 9.1%, and CO_2 – 19.02% ± 4.14% (Figure 8).

2. Addition of *Saccharomyces pastorianus* (Stage IV)

(1) In Stage IV, the addition of *S. pastorianus,* the average COD removal rate was 98.72 % ± 1.8 %, increasing the average COD removal rate by 3.02%.

(2) In Stage IV, the average gas makeup was N_2 – 2.52% ± 3.56%, CH_4 – 71.39% ± 1.94%, and CO_2 – 25.17% ± 0.32%. CO_2 experienced an increase of 6.15%, which indicates the presence and adaptation of *S. pastorianus* in the ABR. The percentages of CH_4 increased by 8.66%, which indicates that *S. pastorianus* was able to promote methanogenesis and increase total CH_4 production (Figure 8).

3. Addition of *Saccharomyces cerevisiae* (Stage II)

(1) In Stage II, the average COD removal rate was 96.76 % ± 2.6 %, increasing the average COD removal rate by 1.06%.

(2) In Stage II, the average gas makeup was N_2 – 14.49% ± 8.63%, CH_4 – 62.73% ± 6.5%, and CO_2 – 23.4% ± 2.9%. CO_2 experienced an increase of 4.38%, which indicates the presence and adaptation of *S. cerevisiae* in the ABR, as CO_2 is the gas product of *S. cerevisiae* (Figure 9). Although the percentages of CH_4 did not change, remaining at 62.73%, the standard deviation of CH_4 is less in Stage II, which indicates improved stabilization of gas production after the addition of *S. cerevisiae.*

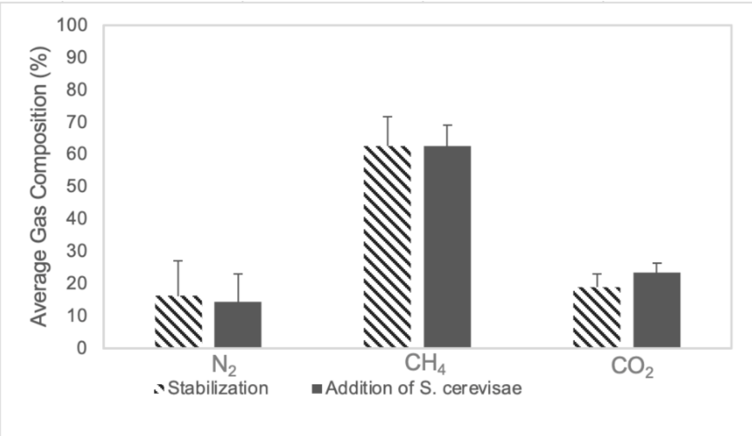

Figure 9. Average Gas makeup of Stage I and Stage II There is a greater gas percentage in CO_2, same gas percentage in CH_4, and less gas percentage in N_2 in Stage II.

4. Comparison of between *Saccharomyces pastorianus* and *Saccharomyces cerevisiae*

Both additions of yeast were able to increase the average COD removal rate range as well CO_2 content, displaying yeast's ability to fermentation and therefore aid in the anaerobic reactions occurring in the ABR. It was observed, however, that *S. pastorianus* exhibited indications of fermentation aid more prominently, as CH_4 content was higher than *S.*

cerevisiae by 8.66% (Figure 10); furthermore, the COD removal rate is also higher by 1.96% (Figure 11).

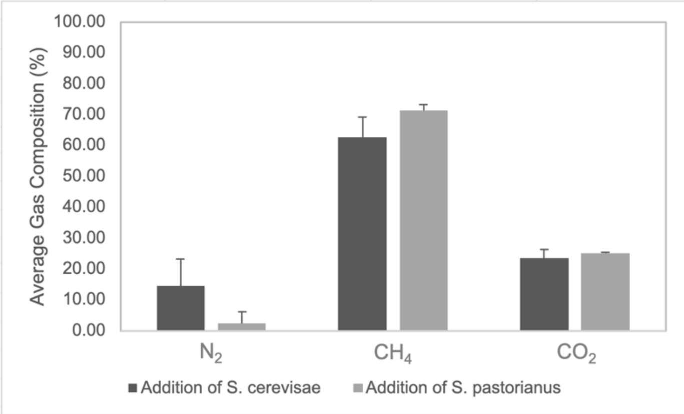

Figure 10. Comparison of Gas Makeup between *Saccharomyces cerevisiae* and *Saccharomyces pastorianus* *S. pastorianus* has higher percentages of CH_4 and CO_2, and lower percentage of N_2.

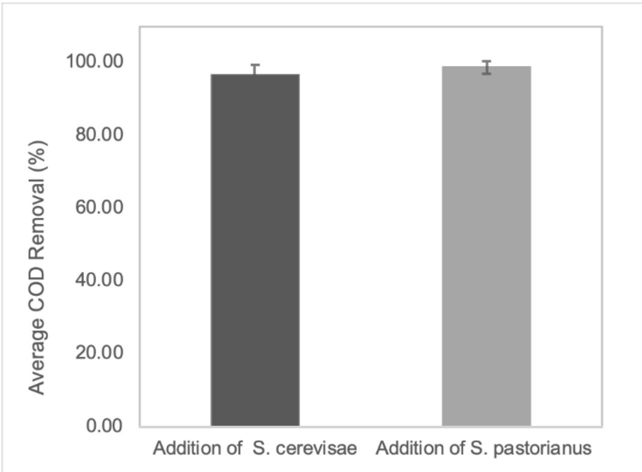

Figure 11. Comparison of Average COD removal rate between *Saccharomyces cerevisiae* and *Saccharomyces pastorianus* *S. pastorianus* has a higher COD removal rate.

5. Rinsing of *S. pastorianus* and *Saccharomyces cerevisiae* (Stage III and Stage V)

(1) Throughout Stages II and IV, the COD removal rates were greater than that of Stage I, the stabilization period. It is expected Stage III, the rinsing of *S. cerevisiae*, and Stage V, the rinsing of *S. pastorianus* to have the opposite effect, drastically decreasing the COD removal rate. This is based on the idea that rinsing will rinse out the majority of the added yeast population, leaving an extremely small value of yeast in the ABR bio plates.

However, in Stage III, the COD removal rate did not decrease as expected, yielding an average COD removal rate of 95.51±4.5%. Furthermore, the lowest effluent COD concentration observed was 0 mg/L, which appeared multiple times throughout Stage III.

Though Stage I does have a higher COD removal rate of 95.70 % ± 1.4%, the lowest effluent concentration observed was 29mg/L. The greater standard deviation in Stage III is an anticipated result, as the rinsing of microorganisms in general should contribute to sudden peaks in effluent COD concentration. However, the overall COD removal rate of Stage III is impressive, and suggests that there are quite significant values of the *S. cerevisiae* remnant population in the ABR.

A similar trend was observed in Stage V, the rinsing of *S. pastorianus.* The average COD removal rate in Stage V was 97.09 % ± 2.0 %. Though this COD removal rate is lower than the COD removal rate of Stage IV, it is higher than the original COD removal rate of Stage I (95.70 % ± 1.4%), indicating that there should be remnants, at a sufficient population, of both *S. pastorianus* and *S. cerevisiae* in the ABR. Table 3 presents the information described.

(2) The gas production did decrease, however, during Stage III and Stage IV. This suggests that rinsing overall does affect the gas production of the ABR, however, does not significantly affect the yeast populations in the ABR. Table 4 presents the information described.

Table 3. Average COD removal rate and various effluent concentrations throughout extended periods with varying yeast variables

Stage	Yeast Variables	COD_{inf} (mg/L)	Lowest COD_{ef} (mg/L)	Highest COD_{ef} (mg/L)	Average COD_{ef} (mg/L)	Average COD Removal
I	-	850 -1200	29	47	39.8	95.70% (±1.4%)
I'	-	850 -1200	52	100	70.67	88.80% (9.6%)
II	+ *S. cerevisae*	850 -1200	4	84	35.8	96.76% (±2.6%)
II'	+ *S. cerevisae*	850 -1200	52	84	64.67	93.44% (±2.2%)
III	- *S. cerevisae*	850 -1200	0	63	29.6	95.51% (±4.5%)
III'	- *S. cerevisae*	850 -1200	26	36	31.5	93.21% (±4.5%)
IV	+ *S. pastorianus*	850-1200	0	64	25.4	98.72% (±1.8%)
IV'	+*S. pastorianus*	850 -1200	21	33	26.75	96.96% (±1.8%)
V	- *S. pastorianus*	850 -1200	8	46	24	97.09% (±2.0%)
V'	- *S. pastorianus*	850 -1200	27	70	38.75	96.25% (±1.9%)

Table 4. Average makeup of gas throughout extended periods with varying yeast variables

Yeast Variables	Average N_2 Gas	Average CH_4 Gas	Average CO_2 Gas
Stabilization (Stage I)	16.2% (±10.93%)	62.73% (±9.1%)	19.02% (±4.14%)
Addition of *S. cerevisae* (Stage II)	14.49% (±8.63%)	62.73% (±6.5%)	23.4% (±2.9%)
Rinsing of *S. cerevisae* (Stage III)	27.71% (±3.17%)	58.4% (±6.3%)	16.5% (±3.6%)
Addition of *S. pastorianus* (Stage IV)	2.52% (±3.56%)	71.39% (±1.94%)	25.17% (±0.32%)
Rinsing of *S. pastorianus* (Stage V)	19.96% (4.93%)	58.72% (±9.9%)	22.08% (±2.8%)

(三) Addition of *Saccharomyces cerevisiae* and *Saccharomyces pastorianus* into ABR environment at low temperature

1. Addition of *Saccharomyces pastorianus* (Stage IV')

In Stage I', the stabilization of the ABR, the average COD removal rate was 88.0 % ± 9.6% while in Stage IV', the addition of *S. pastorianus,* the average COD removal rate was 98.72 % ± 1.8 %. Similar to Stage IV compared to Stage I, Stage IV' had a greater COD removal rate, suggesting that the addition of *S. pastorianus* exhibits the same trend in terms of improving COD removal rate disregarding environmental temperature differences. Figure 12 presents the information described.

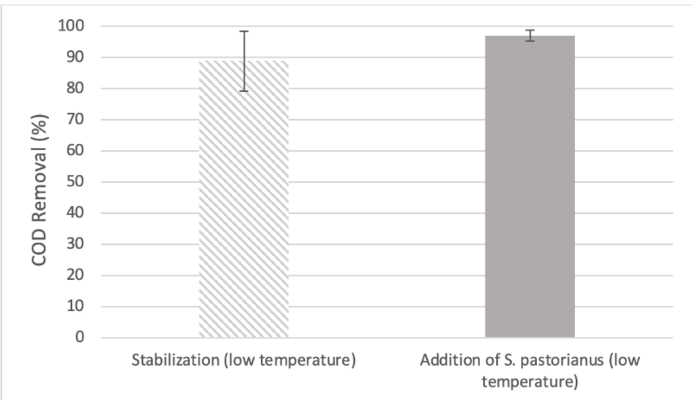

Figure 12. Average COD removal rate of Stage I' and Stage IV' Stage IV' has a greater COD removal rate than Stage I'.

2. Addition of *Saccharomyces cerevisiae* (Stage II')

In Stage I', the stabilization of the ABR, the average COD removal rate was 88.0 % ± 9.6% while in Stage II, the addition of *S. cerevisiae,* the average COD removal rate was 93.44 % ± 2.2 %. Figure 13 presents the information described.

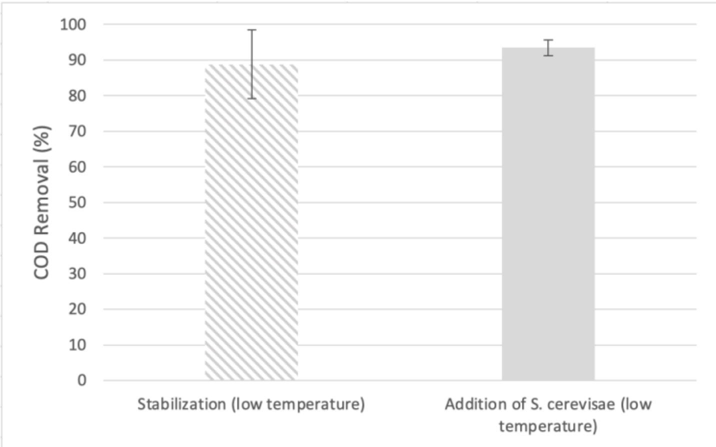

Figure 13. Average COD removal rate of Stage I' and Stage II' Stage II' has a greater COD removal rate than Stage I'.

3. Comparison of between *Saccharomyces cerevisiae* and *Saccharomyces pastorianus* at high and low temperatures

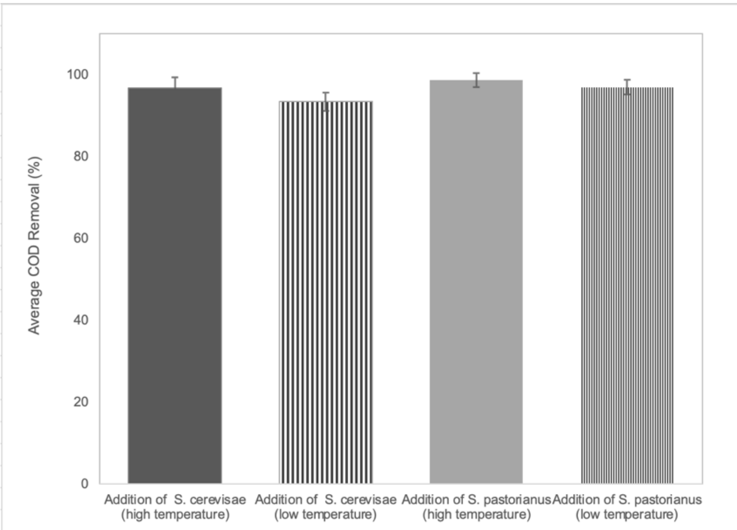

Figure 14. Comparison of Average COD removal rate between *Saccharomyces cerevisiae* and *Saccharomyces pastorianus* at high and low temperatures

Similar to Stage II compared to Stage I, Stage IV' had a greater COD removal rate, suggesting that the addition of *S. pastorianus* exhibits the same trend in terms of improving COD removal rate disregarding environmental temperature differences. To observe whether the addition of yeast will help raise the overall temperature of the anaerobic immobilized bioreactor. Figure 14 presents the information described.

To observe whether the addition of yeast will help raise the overall temperature of the anaerobic immobilized bioreactor

三、ABR Temperature under Controlled Psychrophilic Conditions

(一) ABR temperature raise

The thermodynamics equation (equation 2), where T: Temperature of the mixed fluids, m1: Mass of liquid within ABR, T1: Temperature of mass of liquid within ABR, m2: Mass of inflow liquid, T2: Temperature of mass of inflow liquid, calculates the theoretical temperatures of the ABR.

$$T = (m_1c_1T_1 + m_2c_2T_2) / (m_1c_1 + m_2c_2) \qquad \text{(equation 2.)}$$

1. **<u>Addition of *Saccharomyces pastorianus*</u> under Controlled Psychrophilic Conditions– Stage IV' (Figure 15.)**

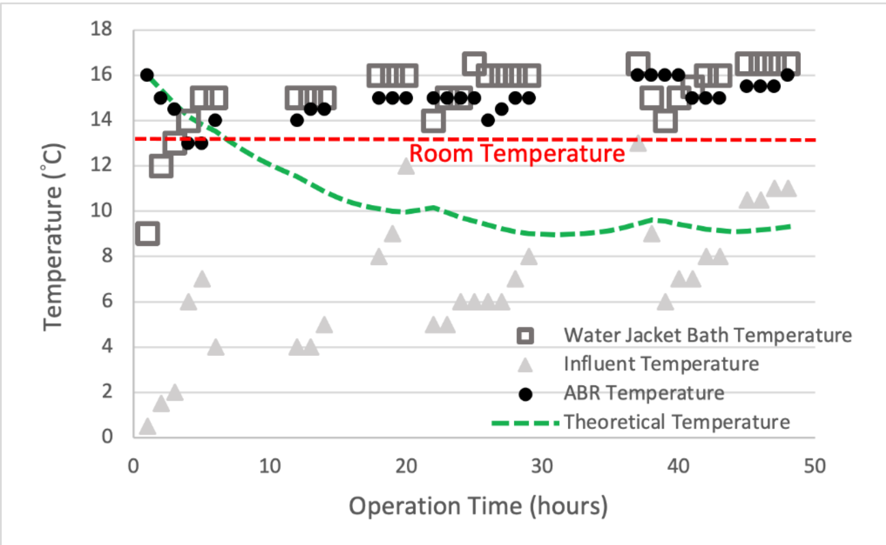

Figure 15. Comparison of Calculated ABR Temperature and actual ABR Temperature – Stage IV'

(1) The ABR temperature diverges from the theoretical ABR temperature at 6 operation hours. From there, the ABR temperature gradually rises, and at the 48^{th} operation hour, a 6.69°C difference from the theoretical temperature is reached.

(2) The average temperature difference was 4°C. The temperature difference can be attributed to the heat generated through fermentation, as *S. pastorianus*'s optimal fermentation temperature ranges between 8°C to 14°C, and within that range, should be able to generate heat that can increase the surrounding environment.

2. **<u>Addition of *Saccharomyces cerevisiae*</u> under Controlled Psychrophilic Conditions– Stage II' (Figure 16.)**

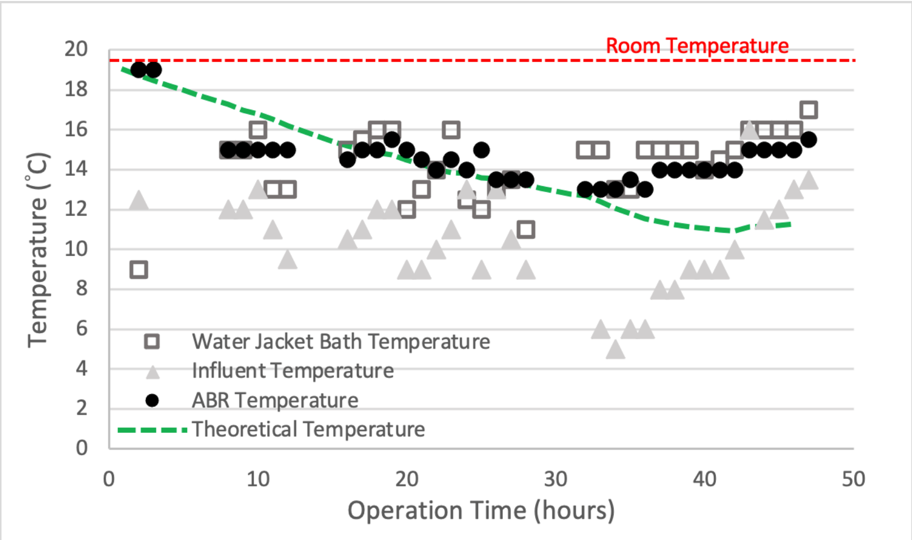

Figure 16. Comparison of Calculated ABR Temperature and actual ABR Temperature – Stage II'

(1) The ABR temperature diverges from the theoretical ABR temperature at 21 operation hours. From there, the ABR temperature gradually rises, and reaches a maximum 4.22°C difference from the theoretical temperature.

(2) The average temperature difference was 2.36°C. This temperature difference indicates that the heat generated from *S. cerevisiae*'s fermentation was effective in raising the ABR temperature, which correlates to *S. cerevisiae*'s ability to generate heat under psychrophilic conditions roughly 3°C.

(3) **Comparison of between the <u>addition of *S. pastorianus*</u> and <u>*S. cerevisiae*</u>**

Both the addition of *S. pastorianus* and *S. cerevisiae* were able to raise the ABR temperature. However, *S. pastorianus* did exhibit a more prominent temperature raise, which ties into *S. pastorianus*'s psychrophilic optimal fermentation rate. The comparison between the two yeast strains suggest that yeast with optimal fermentation temperatures similar to their surrounding environment yield a higher heat raise potential.

3. **<u>Rinsing *Saccharomyces cerevisiae*</u> under Controlled Psychrophilic Conditions– Stage III (Figure 17.)**

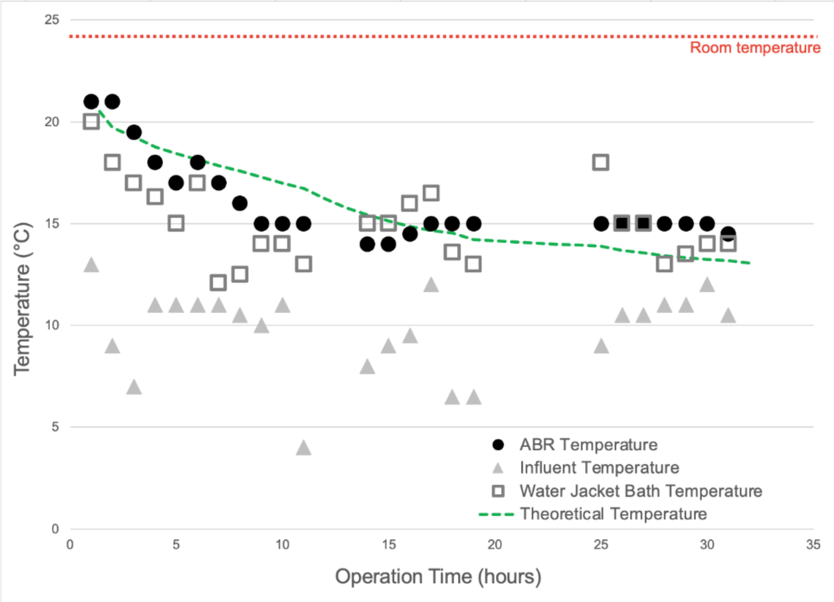

Figure 17. Comparison of Calculated ABR Temperature and actual ABR Temperature – Stage III

(1) The ABR temperature diverges from the theoretical ABR temperature at 12 hours of operation, and had an average temperature difference of 1.26°C and a maximum temperature difference of 1.75°C.

(2) **Comparison of between the <u>addition of *S. cerevisiae*</u> and <u>rinsed *S. cerevisiae*</u>**

Both <u>the addition of *S. cerevisiae*</u> and <u>rinsed *S. cerevisiae*</u> were able to raise the ABR temperature from the theoretical ABR temperature, however, the <u>addition of *S. cerevisiae*</u> was able to raise the ABR temperature an average of 1.1°C more than <u>rinsed *S. cerevisiae*,</u> indicating that the higher the yeast population, the more effective the ability to raise temperature. This trend is also displayed in the <u>rinsed *S. pastorianus*,</u> where the <u>addition of *S. pastorianus*</u> was able to raise the ABR temperature more significantly.

4. <u>Rinsing *Saccharomyces pastorianus*</u> **under Controlled Psychrophilic Conditions–Stage IV' (Figure 18.)**

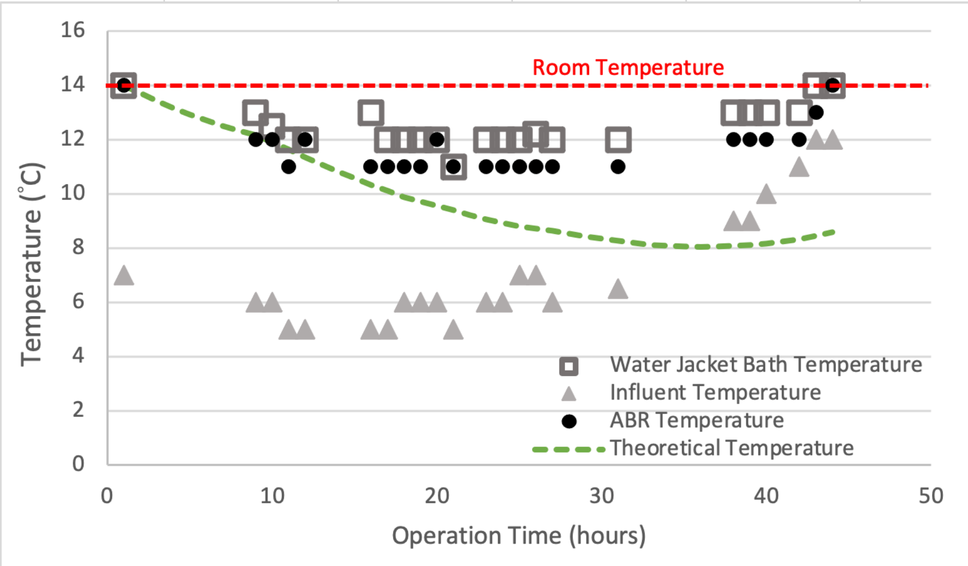

Figure 18. Comparison of Calculated ABR Temperature and actual ABR Temperature – Stage IV'

(1) The ABR temperature diverges from the theoretical ABR temperature and from there, the ABR temperature gradually rises, reaching a maximum 5.4°C difference from the theoretical temperature and an average 2.64°C difference.

(2) **Comparison between the <u>addition of *S. pastorianus*</u> and <u>rinsed *S. pastorianus*</u>**

The <u>addition of *S. cerevisiae*</u> was able to raise the ABR temperature an average of 1.36°C more than <u>rinsed *S. cerevisiae.*</u>

(二) ABR COD Removal Rate and Effluent Concentration in Correlation to ABR Temperature

1. <u>Addition of *Saccharomyces pastorianus*</u> **under Controlled Psychrophilic Conditions–Stage IV' (Figure 19.)**

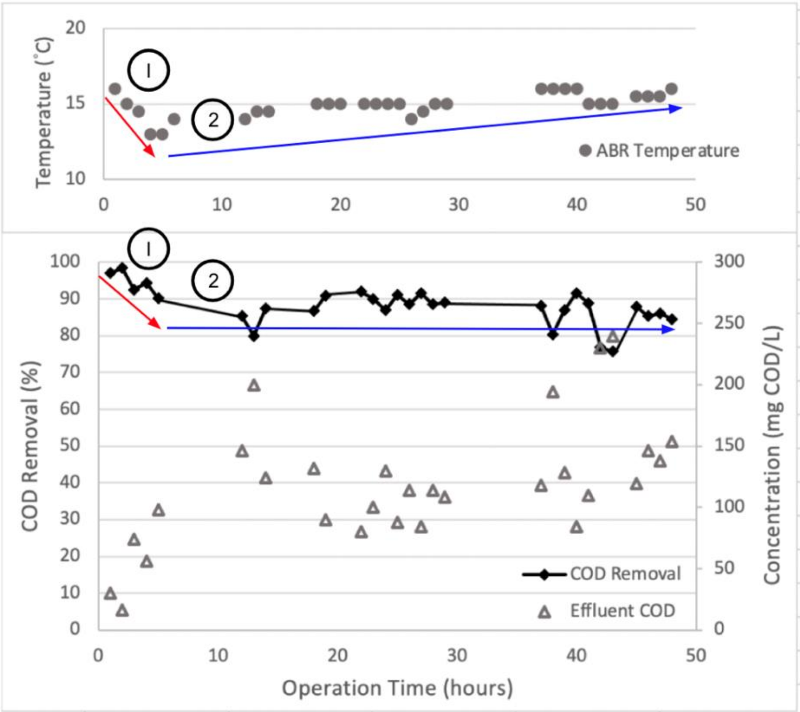

Figure 19. COD Removal Rate and Effluent COD under Psychrophilic Conditions for 48 hours – At the end of Stage IV'

(1) There are two major trends exhibited. The first trend shows a decrease in ABR temperature along with a COD removal rate decrease, which can be correlated to the effects of the psychrophilic environment on the ABR, as the temperature of whole ABR system was abruptly brought to a psychrophilic temperature, reducing the metabolic reactions occurring within the ABR. The second trend shows an increase in ABR temperature and a vibrational period in COD removal rate, where the COD removal rate is gradually reaching stability. The vibrational period could be correlated yeast fermentation of *S. pastorianus* taking place, as the temperature had stopped decreasing, stabilized, and gradually rose. The heat generated from *S. pastorianus* fermentation was able to raise the temperature of the system. Similar trends are exhibited throughout all psychrophilic experiments.

(2) The average COD removal rate during the vibrational period (trend 2) was 86.68%, which nears the average COD removal rate of I' (88%), therefore indicating that COD removal rates at psychrophilic temperatures with the addition of *S. pastorianus* are recovering. Furthermore, the temperature rose from its lowest, 13°C to 16.5°C, indicating that *S. pastorianus* was able to raise the ABR temperature.

(3) Comparison to Hung's (Hung,2019) Experiment

Hung, in his experiment, did not add any yeast nor introduce new microorganisms into his ABR. The addition of *S. pastorianus* had a 9.99% higher COD removal rate than Hung's experiment data, indicating that yeast fermentation is occurring and therefore increasing the metabolic activity within the ABR under psychrophilic temperatures. All comparisons to Hung's experiment are displayed in table 5.

Table 5. Comparison of Stage II' and Stage IV' to literature

Group	Inflow Temperature	Average N_2 Gas (%)	Average CH_4 Gas (%)	Average CO_2 Gas (%)	Average COD Removal Rate (%)
(Hung, 2019)	20°C	23.5 (±5.4)	58.9 (±3.6)	15.4 (±3.5)	76.9
Stage II'	10.25°C	49.86	34.28	15.86	83.65
Stage IV'	8.4°C	55.91	29.53	14.56	86.68

2. Addition of *Saccharomyces cerevisiae* under Controlled Psychrophilic Conditions– Stage II' (Figure 20.)

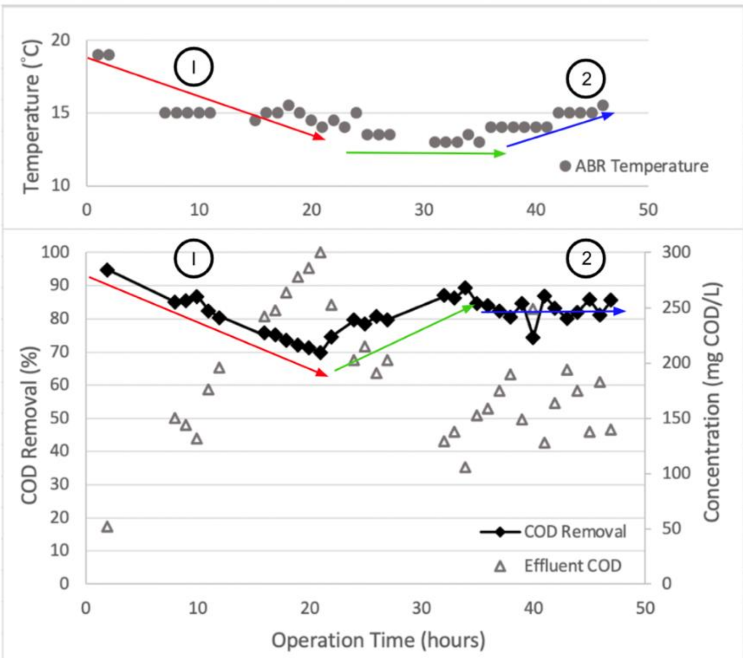

Figure 20. COD Removal Rate and Effluent COD under Psychrophilic Conditions for 48 hours – At the end of Stage II'

(1) The temperature equilibrium and corresponding COD removal rate increase between the trend 1 and trend 2 is a result of *S. cerevisiae*'s lower fermentation rate under psychrophilic temperatures. Although fermentation is taking action, as seen from the COD removal rate increase, the heat generated is not sufficient to immediately experience the temperature increase in trend 2. Nevertheless, trend 2 is still reached, and the ABR temperature is raised.

(2) The average COD removal rate during the vibrational period (trend 2) is 83.65%; the COD removal rate is still high, with only a 4.35% difference from Stage I', and as it is experiencing an ongoing recovery, it is only anticipated to continue increasing. Furthermore, the temperature rose from its lowest, 13°C, to 15.5°C, indicating that *S. cerevisiae* was able to raise the ABR temperature.

(3) Comparison to (Hung, 2019) psychrophilic results and <u>addition of *Saccharomyces pastorianus*</u>

Similar to the difference in temperature raise, the **<u>addition of *S. pastorianus*</u>** was able to yield a 3.03% higher average COD removal rate, as well as a 1°C higher temperature raise. Furthermore, in comparison to Hung's experiment, the COD removal rate was 6.85% higher.

(三) ABR Gas Analysis

All the gas analysis data described are presented in Table 6.

Table 6. Gas makeup under Psychrophilic controlled

Psychrophilic Variables	N_2 Gas	CH_4 gas	CO_2 gas
+ *S. cerevisae (from stage II')*	49.86%	34.28%	15.86%
-*S. cerevisae (from stage III)*	-	-	-
+ *S. pastorianus (from stage IV)*	27.05%	57.10%	16.18%
+ *S. pastorianus (from stage IV')*	13.94%	61.79%	25.11%
- *S. pastorianus (from stage V')*	55.91%	29.53%	14.56%

1. At the end of Stage IV' – <u>Addition of *Saccharomyces pastorianus*</u>

(1) Throughout 48 operation hours, 11 liters of gas were collected. In addition, the effluent contained dissolved gas, indicating that the gas produced was sufficient. Through gas analysis, the average gas makeup of the collected gas was as follows:

N_2 - 13.94%, CH_4 - 61.79%, CO_2 – 25.11%.

(2) Comparison to (Hung, 2019) psychrophilic results

Hung yielded a CH_4 content of 58.3%, therefore, in comparison, the addition of *S. pastorianus* increased CH_4 content by 3.49%. Furthermore, Hung yielded a CO_2 content of 15.4%, therefore, *S. pastorianus* fermentation is indicated as the addition of *S. pastorianus* increased CO_2 content by 9.71%.

2. At the end of Stage II' – Addition of *Saccharomyces cerevisiae*

(1) Throughout 48 operation hours, 9 liters of gas were collected. In addition, the effluent contained dissolved gas, indicating that the gas produced was sufficient. Through gas analysis, the average gas makeup of the collected gas was as follows:

N_2- 49.86%, CH_4- 34.28%, CO_2 – 15.86%.

(2) Comparison to (Hung, 2019) psychrophilic results and addition of *Saccharomyces pastorianus*

As mentioned in the ABR temperature rise and average COD removal rate trends, the addition of *S. cerevisiae* had less fermentative activity than the addition of *S. pastorianus.* This is also indicated by the CO_2 content, where the addition of *S. pastorianus* was 9.25% higher. However, there was still fermentation occurring, but at a considerably slow rate, as the CO_2 content was still 0.46% higher than Hung's CO_2 content. The decrease in CH_4 content can be correlated to the increase in production of H_2, as yeast fermentation aids in the creation of H_2, and H_2 is incapable of detection in the Gas detector (GC 2010 Plus-TCD). The average COD removal rate (83.65%) too indicates that fermentation is aiding in the anaerobic reactions occurring under psychrophilic temperatures.

3. At the end of Stage III – Rinsing of *Saccharomyces cerevisiae*

(1) Throughout the 36 hours, no gas was collected. However, the effluent contained dissolved gas, indicating that there was metabolic activity occurring in the ABR.

(2) Comparison of between the addition of *Saccharomyces cerevisiae* and rinsed *Saccharomyces cerevisiae*

The addition of *S. cerevisiae* yielded 9 liters of gas, therefore indicating that a higher population of *S. cerevisiae* was significantly higher in fermentative activity, and rinsed *S. S. cerevisiae* was not sufficient for aid in psychrophilic anaerobic reactions.

4. At the end of Stage V' – Rinsing of *Saccharomyces pastorianus*

(1) Throughout 48 operation hours, 6 liters of gas were collected. In addition, the effluent contained dissolved gas, indicating that the gas produced was sufficient. Through gas analysis, the average gas makeup of the collected gas was as follows:

N_2- 55.91%, CH_4- 29.53%, CO_2 – 14.56%.

(2) Comparison between the addition of *Saccharomyces pastorianus* and rinsed *Saccharomyces pastorianus*

The addition of *S. pastorianus* yielded a 10.55% higher CO_2, indicating that the population of rinsed *S. pastorianus* was not sufficient for aid in psychrophilic anaerobic reactions.

肆、結論與應用

一、Conclusion

(一) To observe whether the addition of yeast will help raise the overall temperature of the anaerobic immobilized bioreactor.

The addition of yeast under psychrophilic temperatures aided the ABR in raising temperature, as the ABR temperature had an average of 2.36-4°C difference and had a maximum of 4.22-6.69°C difference from the theoretical temperature. Furthermore, the temperature would always rise to above 15°C by the end (around 40 hours) of the experiment. Along with the temperature rise is a vibrational period in COD removal rate, where the COD removal does not decrease, rather, fluctuates within a certain range and displays a gradual turn towards stabilization. The average COD removal rate during the vibrational period was 83.65-86.68%, which, compared to Hung's experiment (Hung,2019), yielded a 6.96-9.99% increase. Compared to the stage I', which was conducted with no psychrophilic temperature influence, the average COD removal rate displayed a trend of recovery, displaying only a 2.12-5.15% difference. Along with COD removal rate increase and recovery, the addition of yeast also aided in gas production, as 9-11 liters of gas were produced, with a 9.71% CO_2 increase, compared to Hung's experiment, that indicates yeast fermentation activity under psychrophilic temperatures. The CH_4 content also increase by 2.9%, too indicating that the addition of yeast aided in methanogenesis. The increase in COD removal rate and gas production and content implies that it was the energy from yeast fermentation that warmed the surrounding environment.

(二) To observe whether the addition of yeast will contribute to gas composition by raising CO_2 and CH_4 levels, and in correspondence, increase COD removal rate.

Under mesophilic conditions, the wastewater treatment was already at a steady and high level. Nonetheless, the addition of yeast promoted COD removal rates by 1.06- 3.02%, with an increase in CO_2 by 4.38-6.15%, indicating fermentative activity, along with a 0-8.66% increase in CH_4.

二、Application

From this study, we have shown that yeast fermentation has potential in facilitating anaerobic reactions, especially under psychrophilic conditions, by increasing the ABR temperature, promoting COD removal rates and gas production, particularly of methane. We hope that this study can be put into application in high latitude countries to improve and enhance the widespread use of Anaerobic Biological Wastewater Treatment. According to specific calculations of a certain sewage treatment plant in Taipei, if said sewage treatment plant were to switch for the traditional aerobic to anaerobic wastewater method of treatment, approximately 742,000 NTD could be saved a day, therefore 22,264,200 NTD could be saved a month, as the anaerobic wastewater method of treatment saves about 1.484 NTD/kWh.

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【評語】200006

本研究藉由酵母的添加，使厭氧生物反應系統可在低溫環境中作用，並藉以提升酵母發酵數近酸化反應，提升甲烷的產率。由於生物反應系統為一複雜的系統，建議應針對厭氧生物反應系統植種，環境的監控(如溶氧)加以討論。