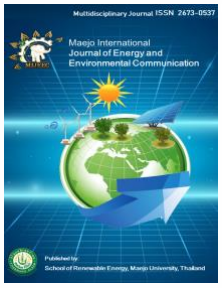
Maejo International Journal of Energy and Environmental Communication

Journal homepage: https://ph02.tci-thaijo.org/index.php/MIJEEC



ARTICLE

Sustainable biobutanol production from *Amorphophallus* tuber starch via optimized ABE fermentation

Solaiyammal Pandi[1], Rajeswaran Ramaraj[2], Prakash Bhuyar[1], Rameshprabu Ramaraj[2], Yuwalee Unpaprom[3,*]

[1]International College, Maejo University, Chiang Mai, 50290, Thailand
[2]School of Renewable Energy, Maejo University, Chiang Mai, 50290, Thailand
[3]Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai, 50290, Thailand

ARTICLEINFO

Article history:
Received 21 November 2024
Received in revised form
19 December 2024
Accepted 26 December 2024

Keywords:
Amorphophallus tuber starch
Clostridium acetobutylicum
Renewable carbon substrate
Batch fermentation
Biobutanol

ABSTRACT

This study examines *Amorphophallus* tuber starch as a renewable carbon substrate for batch fermentation of acetone, butanol and ethanol (ABE) using *Clostridium acetobutylicum* TISTR. Scientists examined spent yeast extract as a potential economic substitute for conventional yeast extract as a nitrogen source. Batch fermentation of C. acetobutylicum required analysis of essential process parameters such as substrate concentration and nitrogen source selection and carbon source type, and pH values. The utilization of gelatinized *Amorphophallus* tuber starch by C. acetobutylicum led to a total solvent output of 28.45 g/L under uncontrolled pH conditions, which is similar to the 30.51 g/L solvent concentration attained with glucose. The highest solvent yield was observed during pure fermentation of gelatinized starch because enzymatic pretreatment failed to boost production, but acidic hydrolysis reduced solvent levels by 22.66%. Acidic conditions at pH 5.5 supported maximum solvent concentration (32.13 g/L), but pH 5.25 produced the highest acetone levels (6.59 g/L). The fermentation process at pH values above six promoted acidogenic pathways while producing minimal solvent amounts. Total solvent production reached its highest level of 28.56 g/L when the initial starch concentration was set at 70 g/L, yet lower concentrations under 30 g/L resulted in acidogenic metabolism. Recycled brewer's yeast extract produced solvents at a level that matched the performance of regular yeast extract by reaching 18.46 g/L. The findings from this research show that Amorphophallus tuber starch can effectively produce biobutanol with nutrients derived from brewery waste operations while supporting sustainable manufacturing approaches based on bio-circular economy sources.

1. Introduction

The fast progression of climate change stems from greenhouse gas (GHG) emissions that occur when fossil fuels burn, leading to a significant worldwide threat. The economic base functions through fossil-dependent energy systems that produce major environmental harm along with resource depletion, while creating significant air pollution impacts (Sophanodorn et al., 2022; Taechawatchananont et al., 2024). The need to find clean, sustainable, and secure energy

* Corresponding author.
E-mail address: yuwaleeun@gmail.com ; yuwalee@mju.ac.th (Y. Unpaprom)

solutions became essential as global demand increases due to industrialization, together with population growth and urbanization (Nasution et al., 2024; Onyemowo et al., 2024a, b). This transition accomplishes two major objectives because it decreases climate risks while developing secure, sustainable energy systems that protect the environment. The worldwide energy transformation depends on renewable energy devices that combine solar power with wind power sources and hydro power systems and geothermal energy, and bioenergy systems (Balakrishnan et al., 2023; Nasution et al., 2024). Bioenergy surpasses other energy alternatives because its ability to convert organic resources from agricultural waste and industrial byproducts, and municipal waste into usable energy products stands out as a main advantage (Mejica et al., 2022). The transportation industry depends on biofuels because they serve as necessary components to minimize carbon emissions. Advanced biofuels derived from non-food lignocellulosic biomass emerged because of primary biofuel (bioethanol and biodiesel) issues that included food versus fuel disputes, along with sustainability matters and land-use effects (Khaodee & Chaiworn, 2023).

Biobutanol maintains its status as an outstanding advanced biofuel because its properties at refinery facilities match exactly with those of gasoline (Liu et al., 2022; Nair & Meenakshi, 2022). The production process of biobutanol through ABE fermentation leads to both enhanced energy density besides decreased volatility, and improved behaviour in mixed fuels than ethanol. Extensive alterations of automobiles are unnecessary since this fuel functions in standard engines and utilizes present fuel distribution systems that need minimal adjustments to their infrastructure (Eloka-Eboka & Maroa, 2023). The compound functions as a platform element for generating bio-derived solvents along with polymers and jet fuels, and it maintains productive processes for green chemicals and sustainable transportation fuels. Bio-fuels produced from butanol provide superior specifications than ethanol due to their high 29.2 MJ/L energy density, along with water resistance and affinity to gasoline combustion engines. Biobutanol maintains excellent compatibility with gasoline at raised proportion ratios while retaining optical compatibility with standard engines, thus making it suitable for automotive and aviation perspectives (Nair & Meenakshi, 2022). Through integrated biorefineries, biobutanol production allows manufacturers to develop high-value products reaching beyond transportation needs, which include butyl acrylates and bio-based plastics and jet fuel precursors (Khunchit et al., 2020).

The fermentation process of ABE relies mainly on Gram-positive *Clostridium* species, yet Clostridium acetobutylicum stands above the other species as the primary choice due to its ability to produce spores while fermenting multiple carbohydrates under anaerobic conditions. BEF fermentation introduced its commercial industry in World War I for acetone production yet reestablished its manufacturing role during recent times due to its potential to create sustainable fuels together with environmentally friendly chemicals. Several technical and economic obstacles obstruct large-scale commercial use of the process because low product concentrations combined with high substrate and nutrient costs create challenges, along with the complicated solvent extraction method. Researchers need to find cheap non-edible raw materials that are readily available in agricultural regions for the large-scale commercialization of ABE fermentation to succeed. Various starch-producing operations work with two main product classes, where cereal plants such as wheat and rice, and maize join barley or they use tuber commodities of sweet potato and potato and suran. The starch content of potato reaches 60%, yet sweet potato contains 70% starch, and corn contains 56% starch, while wheat contains 60% starch, with Amorphophallus containing 77.2% starch.

The current research employs suran (*Amorphophallus* spp.) to generate butanol products. The Araceae family of monocotyledonous flowering plants generates flowers through the spadix inflorescence. The collective group known as Arum tribe represents this classification. The 3300 existing species worldwide belong to 105 different genera (Hau and Gralnick, 2007). *A. commutatus* (Schott) Engl (Araceae) stands as a rare cormous herb among plant species. Through disc-diffusion testing, researchers determined the antibacterial properties of extracts derived from tubers using water solution and organic solvent solutions when combating pathogenic gram-negative bacterial strains. All extract solutions displayed different degrees of antibacterial properties, yet the benzene extract maintained the highest antibacterial strength, while petroleum ether showed moderate effectiveness, followed by chloroform and ethyl acetate extracts. The efficiency of aqueous and methanol extracts proved to be minimal (Arya et al., 2010). The medical practice of traditional medicine finds support from the research outcomes of this study.

Biobutanol production takes priority from tuber crops because these plants naturally contain high starch quantities among first-generation feedstocks (Khunchit et al., 2020). The research explores affordable production methods for biobutanol from tuber crops through biotechnological ethanol enhancement techniques for developing the next-generation biofuel industry. *Amorphophallus* spp. tuber has proven to be an efficient biomass material primarily used in Southeast Asian regions, particularly in Thailand, where it leads domestic production. The tuber industry annually produces 30 million tons and provides fermentation-friendly characteristics because its hydrolysis is easy, and it contains high starch content (60–70% dry basis) coupled with low lignin levels. The plant expands on neglected pieces of land because it needs only basic cultivation methods to survive. The production of microbial growth and solvents requires supplemental nitrogen. The high price of yeast extract makes it industrially impractical as a production solution (Garita-Cambronero et al., 2021). The application of spent brewer's yeast as a waste product offers a cost-efficient solution that corresponds with circular economy practices and enables economical savings along with waste materials conversion. Fermentation of ABE requires optimal pH control because this parameter determines when Clostridium species transition from acidogenic to solventogenic metabolic states (Nair & Meenakshi, 2022). The solvent production rates improve alongside microbial viability when operating within pH levels of 5.0 to 6.0 (Johnravindar et al., 2021). The combination of process intensification methods, including fed-batch fermentation, immobilized cells, and ISPR, contributes to enhanced yield alongside efficiency; however, these benefits depend on the availability of durable strains and persistent feedstock supplies.

The production process of biobutanol from integrated systems supports worldwide sustainability targets alongside EU Green Deal goals and U.S. RFS policies, and Thailand's BCG model. The production of biobutanol from local biomass feedstock, together with industrial waste, assists because it supports the implementation of SDG 7 (clean energy) and SDG 13 (climate action). However, the research gaps remain. The evaluation of Amorphophallus spp. starch in combination with spent yeast exists only in a few studies through controlled laboratory experiments. The fermentation output through the combination of pH regulation and nitrogen sources and starch

substrates in *C. acetobutylicum* TISTR 1462 needs additional investigation, together with techno-economic analyses for developing countries. The research analyzes ABE fermentation through *C. acetobutylicum* TISTR 1462 when utilizing Amorphophallus tuber starch alongside spent brewer's yeast while controlling the fermentation environment with pH regulation. The research aims to measure fermentation outputs as well as examine alternative nutrient sources to improve performance alongside optimal pH conditions to create industrial production methods for biobutanol.

2. Materials and Methods

2.1 Microorganism and culture maintenance

The solventogenic microorganism *Clostridium acetobutylicum* TISTR 1462 originated from the Thailand Institute of Scientific and Technological Research (TISTR). The storage solution consisted of 20% (v/v) glycerol combined with spore suspensions to preserve them at –80°C. Cultures were revived for experimental needs through growth in Reinforced Clostridial Medium (HiMedia, India) under anaerobic conditions at 37 °C for 24 hours.

2.2 Preparation of carbon sources

Amorphophallus tubers from the northeastern Thai province of Sakon Nakhon were freshly collected. The preparation process began with tuber washing, followed by peeling and slicing before moving to drying at 60°C until weight stability, then milling, followed by sieving to produce a 0.5 mm particle size powder as shown in Figure 1. The direct fermentation process involved gelatinizing powdered starch through boiling a 10% (w/v) suspension in distilled water for 15 minutes while maintaining continuous stirring before allowing it to cool at room temperature.

For enzymatic hydrolysis, the gelatinized starch was treated with α-amylase (Termamyl®) at 90°C for 1 hour, followed by glucoamylase (AMG®) at 60°C for 3 hours, to convert starch into reducing sugars (glucose and maltose). Acid hydrolysis involved treating a 10% (w/v) starch slurry with 1% (v/v) HCl at 90°C for 90 minutes, followed by neutralization with NaOH and filtration. Commercial glucose (Sigma-Aldrich) and native Amorphophallus tuber starch (food-grade) were also prepared using the same gelatinization method for comparative studies.

2.3 Preparation of nitrogen sources

Brewer's yeast waste generated at a local facility was used to obtain an extract, which underwent processing procedures.

Figure 1. Step-by-Step Processing Stages of *Amorphophallus* Tuber into Fermentable Flour

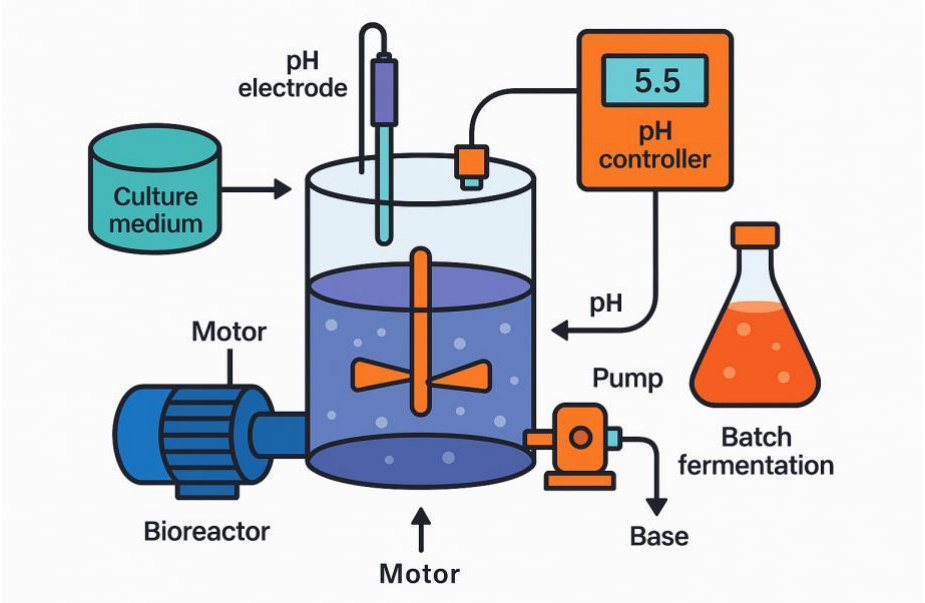


Figure 2. Schematic diagram of the experimental setup for biobutanol production via batch ABE fermentation using *C. acetobutylicum* TISTR 1462

The autolysis process was followed by centrifugation at 6,000 rpm for 15 minutes to remove solids, during which time the collected brewer's yeast biomass was incubated at 50°C because it enhanced amino acid and peptide release. The filtration step followed the storage of the autolysate at 4 ° C for continued use. The control nitrogen source involved Commercial yeast extract obtained from HiMedia Laboratories. The fermentation media received equivalent protein concentrations through the addition of both nitrogen sources at 1.0 g/L.

2.4 Inoculum preparation and batch fermentation conditions

A 100 mL anaerobic serum bottle received 50 mL RCM broth that experienced nitrogen gas purging and was sealed before incubation at 37°C for 24 hours under *C. acetobutylicum* seed culture inoculation. We employed the seed culture to start all batch fermentation experiments through inoculum injection at a concentration of 10% (v/v). A 250 mL serum bottle with 100 mL sterile fermentation medium containing (per liter of solution) KH_2PO_4 0.5 g, K_2HPO_4 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, NaCl 0.5 g, para-aminobenzoic acid 1 mg, biotin 0.5 mg, and thiamine-HCl 1 mg was used for batch fermentations. Amorphophallus starch served as the main the bacterium received its nitrogen supply either through spent brewer's yeast extract or commercial yeast extract solutions. The culture medium required five minutes of nitrogen gas purging and subsequently fifteen minutes at 121°C for autoclaving under anaerobic conditions.

The static fermentations occurred at 37°C for 72 hours while the pH-stat system (Metrohm, Switzerland) maintained pH levels at set points starting from 4.5 to 6.5 by using both 2N NaOH and 2N HCl. The experimental control set utilized fermentations that did not have pH control. The depiction of biobutanol industrial production via batch ABE fermentation by *C. acetobutylicum* TISTR 1462 is shown through Figure 2 and its schematic diagram. The bioreactor system features pH-controlled operations through a combined mechanism, which includes a bioreactor design with parasitic culture medium supply and pH measurement electronics, and base concentration regulation.

2.5 Analytical methods

The evaluation of cell growth occurred through OD_{600} measurements at 600 nm with a Shimadzu UV-1800 UV-Visible spectrophotometer. Measuring residual reducing sugars and starch occurred through the dinitrosalicylic acid (DNS) quantitative method (Miller, 1959). Tests of organic acid and solvent levels used Gas Chromatography with a Flame Ionization Detector (Agilent 7890A) to examine acetone, butanol, ethanol, acetic acid, and butyric acid along with butanol through a 30 m DB-FFAP capillary column (0.32 mm × 0.25 µm). The fermentation used nitrogen as its carrier gas through the process. The instrument operated with detector and injector temperatures set at 250 °C using an oven temperature gradient from 40 °C up to 180 °C at 10 °C/min. The fermentation process was monitored via a pH electrode (Mettler Toledo).

2.6 Statistical analysis

The research experiments happened three times in succession. The researchers present their findings through standard deviation along with mean values. One-way analysis of variance (ANOVA), along with Tukey's HSD post hoc analysis, measured the differences between treatment groups at $p < 0.05$ significance. The data analysis was executed through SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA).

3. Results and Discussion

3.1. Solvent production from various carbon sources

In this experimental study, the fermentation characteristics of *C. acetobutylicum* were evaluated when using uncontrolled pH conditions with various carbon substrates (Table 1). Total solvent production reached 28.45 g/L with Amorphophallus tuber starch when compared to glucose's 30.51 g/L level. *Amorphophallus* starch demonstrates promising potential as an ABE fermentation substrate because it has high starch content and low lignin content and easily passes through gelatinization. The transformation of gelatinized *Amorphophallus* starch to glucose and maltose through enzymatic hydrolysis resulted in decreased solvent production. Experiment tests using fermentation without intermediate steps showed better results than those requiring intermediate steps. The acid-hydrolysis process of cassava starch resulted in a 22.66% decrease in solvent production because furfural and 5-HMF fermentation-inhibiting compounds were formed during the process.

The efficiency evaluation of *C. acetobutylicum* for various carbon sources fermentability under uncontrolled pH conditions determined substrate compatibility for ABE (acetone–butanol–ethanol) fermentation (Capilla et al., 2021). Total solvents production reached 28.45 g/L from Amorphophallus tuber starch substrate tests, while glucose, as the benchmark control, yielded 30.51 g/L. Namely, its low-cost competitive advantages, Amorphophallus starch shows particular suitability for tropical crop-rich regions. The starch content of ~60–70% dry basis, together with minimal lignin presence in *Amorphophallus*, creates optimal conditions for both enzymatic and microbial fermentation of fermentable sugars by simplifying the saccharification-fermentation process.

Table 1. Solvent production from different carbon sources

Carbon Source	Total Solvents (g/L)	Acetone (g/L)	Butanol (g/L)	Ethanol (g/L)
Glucose	30.51	6.12	20.01	4.38
Amorphophallus starch	28.45	5.97	18.41	4.07
Enzymatically hydrolyzed starch	27.94	5.84	18.05	4.05
Acid-hydrolyzed cassava starch	23.0	4.92	15.17	2.91

The use of α-amylase and glucoamylase enzymes for *Amorphophallus* starch gelatinization resulted in minimal variations of the solvents produced compared to standard gelatinized starch fermentation (Kongcha et al., 2022). The results point toward a possible elimination of enzyme pretreatment from processing gelatinized *Amorphophallus* starch while maintaining production equalities, which leads to lower enzyme-based costs. Acid hydrolysis of *Amorphophallus* starch led to the production of fermentable monomers, but it caused a decline in solvent production by 22.66% compared to the initial values. The inhibitory compounds produced

during acid hydrolysis of cassava starch, including furfural and hydroxymethylfurfural, and weak acids, damage both Clostridium viability and metabolic activity (Ray et al., 2024).

The enzymatic saccharification of *Amorphophallus* (konjac) waste achieved better butanol production after alkalioid and oxalate elimination from the waste according to Shao and Chen (2015). The separation of hydrolysis and fermentation (SHF) produced 10.95 g/L total solvents through their configuration, which exhibited greater performance than the 4.3 g/L output achieved without pretreatment. Bioprocess engineering requires specific pretreatment optimization methods because of their importance in achieving superior results (Khunchit et al., 2020). *Amorphophallus* tuber starch processing in its raw form fulfills the requirements of green chemistry and resource efficiency by eliminating unnecessary procedures. The sugar content within *Amorphophallus* spp. along with its availability across Southeast Asia, makes it an ideal candidate for biorefineries (Bhuyar et al., 2022; Hargono et al., 2019), which would lower the need for traditional agricultural feedstocks such as sugarcane and corn.

3.2. Impact of pH regulation on solventogenesis

The optimization of solvent production depended heavily on maintaining the correct pH value (He et al., 2021). A combination of pH 5.5 and 32.13 g/L solvent produced the highest levels at pH 5.5 among the tested values from 4.5 to 6.5 (Table 2). *C. acetobutylicum* needs an exact pH value range to enter its solventogenic metabolic phase. The fermentation process produced more acids than solvents when the pH reached values above 6.0, which resulted in decreased solvent production (da Silva et al., 2022). The study findings agree that lower pH levels trigger the metabolic change toward solventogenesis in *Clostridia*. While the solventogenic *Clostridium* strains fermentation, the pH value determines the metabolic transition between acidogenic and solventogenic processes (Khunchit et al., 2020). Laboratory batch fermenters provided researchers with data about solvent formation levels when pH measurements were adjusted between 4.5 and 6.5 in separate fermentation experiments. Under pH conditions of 5.5, the production of solvent reached its maximum level at 32.13 g/L, whereas acetone yield reached its peak value at 6.59 g/L during conditions at 5.25 (Naleli, 2016). The research indicates that pH levels between 5.0 and 5.5 provide the best conditions for starting and prolonging the solventogenic phase.

The bacterial cells exhibited acidogenic metabolic characteristics when the pH reached values greater than 6.0, which led to increased acetate and butyrate production while solvent formation decreased (Lee et al., 2024). Studies before us show that low pH welcomes increased activation of solventogenic operons (ctfAB, adc and bdhB) while keeping acidogenesis-related pathways suppressed. High pH levels below 4.5 will lead to cell membrane deterioration, together with metabolic blockage, thus highlighting the requirement for accurate pH regulation (Khunchit et al., 2020). Microbial performance remains stable through buffering mechanisms that include $CaCO_3$ together with ammonium acetate, which help stabilize pH fluctuations. The addition of 70 mM ammonium acetate as a buffer enabled Liu et al. (2019) to preserve pH stability and trigger essential solventogenesis gene activations while obtaining results showing 17.8 g/L final solvent yield with 0.35 g/g productivity. The combination of chemical buffering techniques with dynamic pH control demonstrates enhanced fermentation product performance (Liu et al., 2020). Real-time pH regulation controls the outcome of fermentative processes, notably during the important 24–48 hour stage. The new ABE production technology development should include automated pH-stat controls in advanced reactor systems for achieving consistent, scalable processes (Kuila, & Mukhopadhyay, 2023).

Table 2. Effect of pH control on solventogenesis

pH Condition	Total Solvents (g/L)	Acetone (g/L)	Butanol (g/L)	Ethanol (g/L)
Uncontrolled	28.45	5.97	18.41	4.07
4.5	29.1	6.01	18.76	4.33
5.0	30.2	6.33	19.45	4.42
5.25	31.47	6.59	20.21	4.67
5.5	32.13	6.41	21.03	4.69
6.0	14.8	2.1	9.2	3.5
6.5	12.9	1.6	7.8	3.5

3.3. Effect of substrate concentration on solvent yield

The production of *Amorphophallus* starch utilized concentrations which ranged between 35 to 90 g/L. The highest solvent production occurred when using 70 g/L of solvent, which resulted in 28.56 g/L total solvents based on Table 3 findings (Table 3). Acidogenic fermentation occurred when substrate concentration was 35 g/L or below, but fermentation inhibition became prevalent at concentrations above 90 g/L because of either substrate overload stress or increased osmotic pressure. Substrate optimization requires attention because it enables proper microbial stress management alongside proper carbon resource availability (Nandhini et al., 2023). The study evaluated various *Amorphophallus* tuber starch concentrations from 35 to 90 g/L to identify the best level for achieving maximum solvent yield. At 70 g/L substrate concentration, researchers achieved the maximum total solvent production rate, which measured 28.56 g/L, making it the ideal amount of substrate. Cultures used acidogenic pathways to break down carbon when provided with substrate amounts below 35 g/L, and this insufficient energy supply prevented efficient shift to the solventogenic phase (Li et al., 2020). The high osmotic pressure, together with hindered mass transport and elevated solution viscosity caused by concentrations above 90 g/L, resulted in substrate inhibition effects.

Table 3. Influence of substrate concentration on solvent production

Starch Concentration (g/L)	Total Solvents (g/L)	Acetone (g/L)	Butanol (g/L)	Ethanol (g/L)
35	22.5	4.2	14.81	3.49
50	26.8	5.5	17.52	3.78
70	28.56	5.99	18.51	4.06
90	25.7	5.33	16.98	3.39

The experimental results align with models that explain how excessive carbon substrate affects clostridial metabolism by decreasing glycolytic processing while creating metabolic disorders. The successful production of starch-based fermentations demands precise

regulation between carbon feed rate and microbial hydrolytic operations and enzymatic uptake activities. A continuous or fed-batch operational approach would offer a method to introduce substrate at a controlled pace to keep substrate concentrations within their best range (Eloka-Eboka & Maroa, 2023). Deep studies should investigate the combined utilization of mixed carbohydrate feedstocks together with their influence on catabolite repression, since glucose repression remains a major challenge during ABE fermentations. Future improvement in acetone-butanol-ethanol production requires the combination of co-substrate systems and metabolic engineering to deregulate carbon control genes.

3.4. Evaluation of nitrogen sources

The utilization of spent brewer's yeast extract served as a sustainable alternative to nitrogen sources in production. Fermentation based on spent yeast extract enabled the production of 18.46 g/L total solvents, which matched the outcomes from commercial yeast extract (Table 4). The viable nature of spent yeast as a low-cost dietary supplement becomes evident through these findings, while waste valorization and satisfactory fermentation effectiveness remain intact. The implementation of this process at an industrial scale is restricted by its expensive production costs (Khunchit et al., 2020; Liu et al., 2022). The research examined spent brewer's yeast extract (SBY) extracted from brewer's production as a replacement material.

When subjected to fermentation, the total solvent yield from SBY reached 18.46 g/L, showing only a minor decrease compared to the 20.86 g/L obtained with commercial yeast extract. The experimental results establish SBY as a suitable and affordable nitrogen feed for microbe fermentation systems. The dried form (DSY) of SBY consists of at least 45% protein content together with B-complex vitamin constituents, which make it a suitable medium component. The circular economy gains traction from this method because it provides value to industrial waste through reuse. Narueworanon et al. (2020) established that using butanol yields exceeded 11.3 g/L when DSY was combined with sugarcane molasses. The optimization process with response surface methodology identified 0.40 g/g butanol yield by using 50 g/L sugar together with 6 g/L DSY and 6.6 g/L $CaCO_3$. Research data suggests that DSY can serve both as a nitrogen supply and a media stabilizer and demonstrates its potential application areas throughout biofuel production processes. Using SBY as a medium ingredient in fermenters decreases operational expenses while creating sustainable industrial connections that minimize waste outputs. Future research needs to standardize SBY formulation together with nutrient extraction methods and sterilization protocols for maintaining consistent operational results.

Table 4. Effect of nitrogen source on solvent yield

Nitrogen Source	Total Solvents (g/L)	Acetone (g/L)	Butanol (g/L)	Ethanol (g/L)
Commercial Yeast Extract	20.86	4.1	14.02	2.74
Spent Brewer's Yeast Extract	18.46	3.64	12.76	2.06

3.5. Implications and outlook

The results validate *Amorphophallus* tuber starch as an efficient renewable substrate for producing biobutanol through ABE fermentation, according to Table 5. Use of brewery waste nutrients alongside optimized pH management increased fermentation output up to 1.5 times above uncontrolled processes. The production method of biobutanol from local agricultural and industrial residues enables practical decentralized production while complying with the bio-circular-green (BCG) economy standards in tropical areas that have substantial biomaterial availability. Technological improvements need to address continuous production systems and integrated solvent retrieval methods like pervaporation and gas stripping while developing economic models for industrial implementation (Khunchit et al., 2020; Nair & Meenakshi, 2022).

Table 5. Outline of solvent production under various fermentation conditions

Conditions	Total Solvents (g/L)	Acetone (g/L)	Butanol (g/L)	Ethanol (g/L)
Glucose (control, uncontrolled pH)	30.51 ± 0.82	6.12 ± 0.18	20.01 ± 0.57	4.38 ± 0.12
Amorphophallus starch (uncontrolled pH)	28.45 ± 0.74	5.97 ± 0.15	18.41 ± 0.52	4.07 ± 0.09
Enzymatically hydrolyzed starch	27.94 ± 0.63	5.84 ± 0.16	18.05 ± 0.46	4.05 ± 0.08
Acid-hydrolyzed *Amorphophallus* tarch	23.00 ± 0.71	4.92 ± 0.14	15.17 ± 0.48	2.91 ± 0.11
pH-controlled (pH 5.5)	32.13 ± 0.89	6.41 ± 0.20	21.03 ± 0.58	4.69 ± 0.13
pH-controlled (pH 5.25)	31.47 ± 0.85	6.59 ± 0.21	20.21 ± 0.55	4.67 ± 0.14
pH ≥ 6.0	<15.00	-	-	-
Amorphophallus starch	28.56 ± 0.76	5.99 ± 0.17	18.51 ± 0.53	4.06 ± 0.11
Spent brewer's yeast extract	18.46 ± 0.59	3.64 ± 0.12	12.76 ± 0.42	2.06 ± 0.08
Commercial yeast extract	20.86 ± 0.67	4.10 ± 0.13	14.02 ± 0.45	2.74 ± 0.09

These research outcomes confirm that *Amorphophallus* tuber starch, together with spent brewer's yeast extract, present sustainable

foundational material for ABE fermentation development. The optimized conditions enabled substrate optimization and nutrient replacement along with controlled fermentation parameters at pH, which led to a 1.5-fold increase in solvent production. Low-carbon biobutanol production receives practical support from the research findings to establish such systems within areas possessing ample starch-based feedstocks and brewery waste resources (Garita-Cambronero et al., 2021; Liu et al., 2022). The research demonstrates a perfect match with national and international sustainability goals because it unites green raw materials with waste nutrient recycling and implementation of engineering processes (Ramaraj & Unpaprom, 2019). The work aligns specifically with SDGs 7 and 12 and Thailand's BCG economic framework.

Future development should focus on building up processing capacity through continuous fermenter implementation, while including in-process solvent retrieval systems, including pervaporation and gas stripping, and conducting assessments to determine economic and environmental impacts for industrial usability. Genetic work on enhancing *Clostridium* strains, together with complex culture experimentation, will help the microorganisms tolerate substrate and product limitations for increased productivity. This research adds crucial understanding to create self-contained renewable production systems that reduce costs and protect the environment through efficient local resource-based biobutanol production.

4. Conclusion

The combination of *Clostridium acetobutylicum* TISTR 1462 with *Amorphophallus* tuber starch as substrate enables an effective and sustainable biobutanol production through ABE fermentation. Universal usage of gelatinized Amorphophallus starch at 5.5 pH control condition produced optimal results for solventogenesis, which generated 32.13 g/L total solvent and 21.03 g/L butanol concentration. The production of acids through acidogenesis became more favourable when pH levels reached 6.0 or above, which resulted in lower solvent amounts. Fermentation performance with spent brewer's yeast extract as a low-cost by-product proved equivalent to commercial yeast extract, making it a viable, sustainable nitrogen source. The optimal level of starch at 70 g/L produced the highest amount of solvent during fermentation, but other concentrations negatively affected the solventogenesis process. Low-cost feedstocks with industrial residues and optimized fermentation parameters create scalable, decentralized biobutanol production opportunities. The process fits the principles of circular economy while supporting energy security while maximizing waste use, and meeting climate targets. Research efforts must concentrate on maintaining continuous operations, together with in-place solvent collection methods and economic viability analyses for reaching industrial manufacturing potential.

Reference

Balakrishnan, D., Manmai, N., Ponnambalam, S., Unpaprom, Y., Chaichompoo, C., & Ramaraj, R. (2023). Optimized model of fermentable sugar production from Napier grass for biohydrogen generation via dark fermentation. International Journal of Hydrogen Energy, 48(55), 21152-21160.

Bhuyar, P., Shen, M. Y., Trejo, M., Unpaprom, Y., & Ramaraj, R. (2022). Improvement of fermentable sugar for enhanced bioethanol production from *Amorphophallus* spp. tuber obtained from northern Thailand. Environment, Development and Sustainability, 24, 8351-8362.

Capilla, M., San-Valero, P., Izquierdo, M., Penya-roja, J. M., & Gabaldón, C. (2021). The combined effect on initial glucose concentration and pH control strategies for acetone-butanol-ethanol (ABE) fermentation by *Clostridium acetobutylicum* DSM 792. Biochemical Engineering Journal, 167, 107910.

da Silva, D. B., Fernandes, B. S., & da Silva, A. J. (2022). Butanol production by *Clostridium acetobutylicum* DSMZ 792 from cassava starch. Environmental Sustainability, 5(1), 91-102.

Eloka-Eboka, A. C., & Maroa, S. (2023). Biobutanol fermentation research and development: feedstock, process and biofuel production. In Advances and developments in biobutanol production (pp. 79-103). Woodhead Publishing.

Garita-Cambronero, J., Paniagua-García, A. I., Hijosa-Valsero, M., & Díez-Antolínez, R. (2021). Biobutanol production from pruned vine shoots. Renewable Energy, 177, 124-133.

Hargono, H., Jos, B., Kumoro, A. C., & Haryani, K. (2019). Low Temperature Enzymatic Hydrolysis (LTEH) and Fermentation for Bioethanol Generation from Suweg (*Amorphophallus campanulatus* B) Starch. In Journal of Physics: Conference Series (Vol. 1295, No. 1, p. 012035). IOP Publishing.

He, Y., Cassarini, C., & Lens, P. N. (2021). Bioethanol production from H_2/CO_2 by solventogenesis using anaerobic granular sludge: effect of process parameters. Frontiers in Microbiology, 12, 647370.

Johnravindar, D., Elangovan, N., Gopal, N. O., Muthaiyan, A., & Fei, Q. (2021). Biobutanol production from cassava waste residue using *Clostridium* sp. AS3 in batch culture fermentation. Biofuels, 12(10), 1259-1266.

Khaodee, W., & Chaiworn, P. (2023). Sustainable management of rice straw addressing burning issues and harnessing bioethanol potential. Maejo International Journal of Energy and Environmental Communication, 5(3), 43-51.

Khunchit, K., Nitayavardhana, S., Ramaraj, R., Ponnusamy, V. K., & Unpaprom, Y. (2020). Liquid hot water extraction as a chemical-free pretreatment approach for biobutanol production from *Cassia fistula* pods. Fuel, 279, 118393.

Kongchan, W., Unpaprom, Y., Dussadee, N., & Ramaraj, R. (2022). Bioethanol production from low-grade konjac powder via combination of alkaline and thermal pretreatments. Maejo International Journal of Energy and Environmental Communication, 4(3), 27-31.

Kuila, A., & Mukhopadhyay, M. (Eds.). (2023). Production of Biobutanol from Biomass. John Wiley & Sons.

Lee, H. L., Ashoor, S., Yao, Z., & Jang, Y. S. (2024). Characterization of acidogenic phase metabolism in *Clostridium acetobutylicum* ATCC 824 (pCD07239) under different culture conditions. Applied Biological Chemistry, 67(1), 80.

Li, S., Huang, L., Ke, C., Pang, Z., & Liu, L. (2020). Pathway dissection, regulation, engineering and application: lessons learned from biobutanol production by solventogenic clostridia. Biotechnology for biofuels, 13, 1-25.

Liu, H., Zhang, J., Yuan, J., Jiang, X., Jiang, L., Zhao, G., ... & Liu, B. (2019). Omics-based analyses revealed metabolic responses of *Clostridium acetobutylicum* to lignocellulose-derived inhibitors furfural, formic acid and phenol stress for butanol fermentation. Biotechnology for biofuels, 12, 1-20.

Liu, Y., Yuan, Y., Ramya, G., Singh, S. M., Chi, N. T. L., Pugazhendhi, A., ... & Mathimani, T. (2022). A review on the promising fuel of the future–Biobutanol; the hindrances and future perspectives. Fuel, 327, 125166.

Mejica, G. F. C., Unpaprom, Y., Whangchai, K., & Ramaraj, R. (2022). Cellulosic-derived bioethanol from Limnocharis flava utilizing alkaline pretreatment. Biomass Conversion and Biorefinery, 12(5), 1737-1743.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical chemistry, 31(3), 426-428.

Nair, P., & Meenakshi, H. N. (2022). Review on the synthesis, performance and trends of butanol: a cleaner fuel additive for gasoline. International Journal of Ambient Energy, 43(1), 4207-4223.

Naleli, K. (2016). Process modelling in production of biobutanol from

lignocellulosic biomass via ABE fermentation (Doctoral dissertation, Stellenbosch: Stellenbosch University).

Nandhini, R., Rameshwar, S. S., Sivaprakash, B., Rajamohan, N., & Monisha, R. S. (2023). Carbon neutrality in biobutanol production through microbial fermentation technique from lignocellulosic materials–A biorefinery approach. Journal of Cleaner Production, 413, 137470.

Nasution, F., Theanhom, A. A., Unpaprom, Y., Ramaraj, R., Manmai, N., & Chumpookam, J. (2024). *Muntingia calabura* fruits as sources of bioactive compounds and fermentative ethanol production. Biomass Conversion and Biorefinery, 14(4), 4703-4714.

Nguyen, T. V. T., Unpaprom, Y., Manmai, N., Whangchai, K., & Ramaraj, R. (2022). Impact and significance of pretreatment on the fermentable sugar production from low-grade longan fruit wastes for bioethanol production. Biomass Conversion and Biorefinery, 12(5), 1605-1617.

Onyemowo, M., Unpaprom, Y., & Ramaraj, R. (2024a). Exploring the potential of natural dyes in DSSCs: Innovations for efficient light harvesting and charge separation through Co-sensitization. Optical Materials, 148, 114860.

Onyemowo, M., Ramaraj, R., Unpaprom, Y., & Ramaraj, R. (2024b). Sustainable energy development combining natural dyes with titanium dioxide quantum dots in high-efficiency biological solar cells. Energy, Ecology and Environment, 9(4), 331-343.

Ramaraj, R., & Unpaprom, Y. (2019). Enzymatic hydrolysis of small-flowered nutsedge (*Cyperus difformis*) with alkaline pretreatment for bioethanol production. Maejo International Journal of Science and Technology, 13(2), 110-120.

Ray, S., Mukherjee, A., & Ghosh, U. (2024). Advancements in microbial fermentation of agro and food processing wastes for generation of biofuel. Solid-Gaseous Biofuels Production, 245-285.

Shao, M., & Chen, H. (2015). Feasibility of acetone–butanol–ethanol (ABE) fermentation from *Amorphophallus* konjac waste by Clostridium acetobutylicum ATCC 824. Process Biochemistry, 50(8), 1301-1307.

Sophanodorn, K., Unpaprom, Y., Whangchai, K., Duangsuphasin, A., Manmai, N., & Ramaraj, R. (2022). A biorefinery approach for the production of bioethanol from alkaline-pretreated, enzymatically hydrolyzed Nicotiana tabacum stalks as feedstock for the bio-based industry. Biomass Conversion and Biorefinery,12, 891-899.

Taechawatchananont, N., Manmai, N., Pakeechai, K., Unpaprom, Y., Ramaraj, R., & Liao, S. Y. (2024). Potentials of bioethanol production from sunflower stalks: value-adding agricultural waste for commercial use. Biomass Conversion and Biorefinery, 14(11), 11799-11811.