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RESEARCH ARTICLE
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Consolidated Bioprocessing of Tapioca Solid Waste by Microbial Consortium of Baker's and *Tapai* Yeast to Sustainable Bioethanol Production

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ABSTRACT

Tapioca solid waste (TSW), a starchy, fibrous byproduct of the tapioca industry, is widely produced but remains largely underutilized, particularly as a feedstock for renewable energy production such as bioethanol. Given the growing global demand for low-carbon fuels and circular bioeconomy-based waste valorization strategies, this study investigated bioethanol production from TSW using consolidated bioprocessing (CBP) with a microbial consortium of baker's yeast and *tapai* yeast. A factorial completely randomized design (CRD) was implemented, examining three factors: concentrations of baker's yeast, *tapai* yeast, and TSW substrate, each at 5%, 10%, and 15% (w/v). This design enabled evaluation of interactive effects between microbial consortium composition and substrate loading under CBP conditions. Key parameters measured included bioethanol concentration, final pH of the fermentation medium, substrate consumption, bioethanol production efficiency per unit of substrate, theoretical fermentation efficiency, and bioethanol yield. The results showed that all three factors significantly influenced most measured parameters, except final pH, indicating that process performance was primarily governed by microbial–substrate interactions rather than by pH variation. The optimal treatment combination was identified as 10% baker's yeast, 10% *tapai* yeast, and 10% TSW substrate. This optimal condition yielded a bioethanol concentration of 37.15g/L, with a final pH ranging from 3.74 to 4.73, reflecting stable fermentation under mixed amylolytic–fermentative metabolism. Furthermore, it achieved 59.93% substrate consumption, 71.65% bioethanol production efficiency per unit of substrate, 69.32% theoretical fermentation efficiency, and a 4.16% bioethanol yield, demonstrating effective starch hydrolysis and sugar conversion without the addition of commercial enzymes. These findings strongly suggest that CBP using a microbial consortium on tapioca solid waste has significant potential as an effective method for bioethanol production, offering a non-GMO, low-cost, and environmentally sustainable pathway within a circular bioeconomy framework.

Keywords: Tapioca Solid Waste (TSW), Consolidated Bioprocessing, Bioethanol, Microbial Consortium.

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INTRODUCTION

The escalating global demand for energy, driven by increasing human activities, continues to outpace the diminishing reserves of fossil fuels (Broda et al., 2022; Afedzi & Parakulsuksatid, 2023). Concurrently, the extensive reliance on fossil fuels contributes significantly to harmful greenhouse gas emissions, exacerbating environmental concerns. These pressing issues allow an urgent need for the development of sustainable,

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environmentally friendly, and cost-effective alternative renewable energy sources (Erdiwansyah et al., 2024). Globally, bioethanol production has exceeded 110 billion liters per year, dominated by the United States and Brazil, reflecting its strategic role in decarbonizing the transport sector and reducing greenhouse gas emissions (Hoang & Nghiem, 2021). Bioethanol, derived from polysaccharides, has emerged as a promising renewable biofuel, attracting considerable research and development efforts (Broda et al., 2022; Li et al., 2024). However, its widespread production faces significant challenges, particularly regarding the sustainability and economic viability of current technologies. A major concern is the predominant use of corn starch as a feedstock in large-scale bioethanol industries, consuming nearly 30% of global corn production (Gronchi et al., 2022). While this mitigates petroleum dependence, it can lead to increased prices and reduced availability of food and feed commodities, alongside environmental impacts such as water depletion and soil degradation. This competition with food and feed resources raises concerns related to food security, land-use change, and indirect environmental impacts, underscoring the importance of second-generation and waste-based bioethanol feedstocks (Chowdhury et al., 2025).

Consequently, there is a critical need to identify alternative feedstocks that are abundant, cost-effective, and do not compete with the food supply. Tapioca solid waste (TSW), a starchy biomass by-product from cassava processing, presents a compelling alternative, comprising approximately 60% starch and 20% cellulose (Arnata et al., 2021). Cassava processing industries typically generate 20–30% solid waste relative to raw material input, representing a major environmental burden if not properly managed (Oghenejoboh et al., 2021). Despite its potential, TSW is often underutilized, primarily disposed of as animal feed or directly discarded, contributing to environmental pollution. Indonesia, a leading cassava producer, generates 19–21 million tons of cassava annually, yielding 4–6 million tons of TSW per year (Murniati et al., 2021). This abundant, low-cost, and readily available waste stream positions TSW as an ideal, yet underexploited, raw material for bioethanol production. Valorization of this waste into bioethanol aligns strongly with circular bioeconomy principles by transforming low-value residues into renewable energy carriers (Wagh et al., 2024).

A primary hurdle in bioethanol production from starchy biomass is the substantial energy input required for cooking and gelatinization, which elevates temperatures from 32°C to 95°C (Allan et al., 2020). This energy demand can account for 10–20% of the bioethanol fuel value or 6–13% of the total energy consumption in corn starch-based bioethanol production (Robertson et al., 2006). This energy-intensive step significantly reduces net energy efficiency and increases production costs at industrial scale (Schmitt et al., 2025). Furthermore, the high cost of supplying large amounts of enzymes for biomass hydrolysis, particularly during liquefaction and saccharification, remains a major constraint in bioethanol production (Tanimura et al., 2015; Broda et al., 2022). Recent advances in microbial engineering have attempted to overcome this limitation by enabling in situ enzyme production; however, such approaches often rely on genetically modified strains. Although enzymatic hydrolysis has several advantages over acid hydrolysis such as lower environmental impact and reduced by-product formation that can inhibit fermentation the development of a cost-effective ethanol production process with an appropriate energy balance still requires further improvement (Vasi´ et al., 2021). To address this issue, various bioethanol production strategies have been explored, including separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), simultaneous saccharification and co-fermentation (SSCF), non-isothermal saccharification and fermentation (NSSF), and consolidated bioprocessing (CBP) (Liu et al., 2018). Among these, CBP technology is particularly attractive due to its high cost-saving efficiency and effectiveness, integrating enzyme production, polysaccharide saccharification, and sugar fermentation into a single-step process within a single bioreactor (Tanimura et al., 2015). Thus, the one-step process is a promising strategy for cost-effective ethanol production from starchy biomass (Krajang et al., 2021). However, the CBP method requires specific microorganisms that are simultaneously capable of producing hydrolytic enzymes and fermentative properties to produce bioethanol (Schlembach et al., 2024). Most recent CBP developments (2020–2025) emphasize engineered monocultures or synthetic consortia, which may face regulatory, economic, and biosafety constraints (Singhania et al., 2022).

Several researchers have reported the production of bioethanol by the CBP method from various types of raw materials and different microbes, including raw corn starch (200g/L) with cloned and recombinant amylase in *Saccharomyces cerevisiae* Y294, at 30°C for 192 h, producing a bioethanol concentration of 89.35–98.13g/L (Cripwell et al., 2019). Raw starch (200g/L) with *Saccharomyces cerevisiae* expressing α-amylases and glucoamylases at 30°C for 144 h produced bioethanol concentration of 44.77g/L (Sakwa et al., 2018), raw flour (14%w/v) with *Trametes hirsuta* Bm-2 at 32°C for 288 h produced bioethanol concentration of 13g/L (Olguin-Maciel et al., 2019). Raw starch with *Saccharomyces cerevisiae* produced a bioethanol concentration of 0.67g/L (Gronchi et al., 2022). Potato starch (50–150g/L) with metabolically engineered *Bacillus subtilis* at 37°C for 96 h produced a concentration of 16.3–21.5g/L (Maleki et al., 2021). Brewers spent grains lignocellulose (250g/L) with *Aspergillus oryzae* and *Saccharomyces cerevisiae* NCYC479 for 240 h produced a bioethanol concentration of 37g/L (Wilkinson et al., 2017). These studies show that the development of the CBP method for bioethanol production has been carried out using various types of raw materials, with varying process conditions (substrate concentration, temperature, process duration), and producing different bioethanol concentrations. In addition, many of these studies also use genetically modified microbes, which can simultaneously produce amylolytic enzymes and convert them into bioethanol. Although researchers have successfully developed genetically modified microbes, these microbes have the potential to

increase production costs and produce generations of genetically modified organisms or GMOs (Schlembach et al., 2024). In addition, genetically engineered microbes are feared to be potentially harmful if released into the environment (Tanimura et al., 2015; Nuzzo et al., 2020). The use of bacteria in CBP such as *Clostridium* sp. has also been reported to produce many unwanted by-products such as organic acids and nitrogen gas (Dudek et al., 2024). In this regard, researchers are increasingly interested in developing CBP methods using microbes that are cheap, easy to obtain, and environmentally safe. Here, we demonstrate, for the first time, a non-GMO, naturally occurring microbial consortium composed of baker's yeast and tapai yeast capable of performing consolidated bioprocessing of tapioca solid waste without external enzyme addition, thereby addressing a critical gap in sustainable CBP development.

Baker's yeast and *tapai* yeast are potential candidates for developing CBP methods. Baker's yeast and *tapai* yeast are easy to obtain, inexpensive, and environmentally friendly. Baker's yeast has been reported to contain a monoculture of *S. cerevisiae* that is able to convert glucose into bioethanol, while *tapai* yeast contains a multicultural of microbes that have amylolytic and fermentative activities (Arnata et al., 2021). *Tapai* yeast is reported to contain *Amylomyces rouxii*, *Rhizopus oryzae*, *Endomycopsis burtonii*, *Mucor* sp., *Candida utilis*, *S. fibuligera*, *S. cerevisiae* and several lactic acid bacteria such as *Pediococcus pentosaceus*, *Lactobacillus plantarum*, and *L. fermentum* (Sujaya et al., 2011). Our preliminary research successfully isolated amylolytic microbial isolates from *tapai* yeast, exhibiting amylolytic activity ranging from 0.73 to 0.89U/mL and demonstrating their ability to produce bioethanol at concentrations of 0.20–4.70% (v/v) in Peptone Yeast Glucose (PYG) medium (Gunam et al., 2021, Gunam et al., 2023). In addition, bioethanol production reached 28.277±0.228g/L when wild cassava flour was used as the substrate (Gunam et al., 2026). Furthermore, co-fermentation of TSW media yielded 5.75–7.18% v/v bioethanol (Arnata et al., 2021). Despite these promising results, the optimal synergistic concentration of baker's yeast and *tapai* yeast within a CBP framework using TSW media, for simultaneous enzyme production and bioethanol fermentation, remains unknown and unreported.

Consortium synergism in bioethanol production is strongly influenced by process parameters, such as substrate concentration and the concentrations of individual yeast cultures (Mardawati et al., 2019). Substrate concentration, whether too high (leading to increased viscosity and inhomogeneous culture distribution) or too low (resulting in suboptimal microbial growth), can limit final product formation (Voulgaris et al., 2025). Currently, the optimal conditions for bioethanol production via the CBP method using a microbial consortium of baker's yeast and *tapai* yeast with TSW as a feedstock have not been established.

In this study, we aim to eliminate the need for commercial enzymes in the liquefaction and saccharification steps of bioethanol production by leveraging the inherent enzymatic capabilities of baker's and *tapai* yeast. Therefore, this research demonstrates the potential of this microbial consortium for bioethanol production by systematically determining the optimal conditions for substrate, baker's yeast, and tapai yeast concentrations using the CBP method with TSW medium, while elucidating the synergistic effects of microbial composition and substrate loading relevant to industrial scale-up. Bioethanol concentration, efficiency of substrate consumption by microbial cells, bioethanol production per unit of substrate, fermentation efficiency, and production yield were analysed as key performance indicators of the fermentation process.

MATERIALS & METHODS

Materials

The materials used were solid waste from the tapioca industry in Bogor, Indonesia (6.55° S, 106.82° E) was used as the material in this study. The location is characterized by a humid subtropical climate, with average ambient temperatures ranging between 28 and 31°C. Fermifan brand baker's yeast, NKL brand *tapai* yeast obtained from a cake ingredient shop in Denpasar City. The tools used were water bath, micro pipette, spectrophotometer, gas chromatography (GC-Gas Chromatography-Agilent with HP-5 column), distillatory, analytical balance, and glassware.

Experiment Design

This study used Factorial Randomized Complete Block Design (RCBD Factorial) with 3 factors, namely the concentration of liquid culture of *tapai* yeast consisting of 3 levels namely 5% v/v, 10% v/v, and 15% v/v; baker's yeast consisting of 3 levels namely 5% v/v, 10% v/v and 15% v/v and substrate concentration consisting of 3 levels namely 5% w/v, 10% w/v and 15% w/v. The combination of these factors resulted in 27 treatments and each treatment was grouped into 2 (two) based on the bioethanol production time.

Preparation of Baker's Yeast and *Tapai* Yeast Cultures

Isolates of *tapai* yeast and baker's yeast were first propagated in 10mL of potato dextrose broth (PDB) supplemented with yeast extract and grown for 1–2 days to prepare stock cultures. Subsequently, the isolates were transferred to 50 mL of growth medium (in a 200mL Erlenmeyer flask) containing the following composition: glucose (10g/L), yeast extract (1g/L), KH_2PO_4 (0.1g/L), $MgSO_4 \cdot 7H_2O$ (0.1g/L), and $(NH_4)_2SO_4$ (0.1g/L). The cultures were incubated in a shaker at 125rpm and 30°C for 24 h. Microbial counts for both *tapai* yeast and baker's yeast were determined using the total plate count (TPC) method at two time points: the start of fermentation (24 h) and the end of fermentation (96 h).

Bioethanol from TSW Substrate

The TSW was sun-dried to approximately 12% moisture content, milled, and sieved through a 40-mesh screen. The resulting TSW flour was weighed and suspended at concentrations of 10%, 20%, and 30% w/v. The suspensions were gelatinized by heating at 100°C for 10min and then used as the fermentation substrate.

Batch fermentations were conducted in 250mL Erlenmeyer flasks containing 200mL of substrate. Prior to inoculation, the substrate was sterilized at 121°C for 15min, and the pH was adjusted to 5.0 using 1N NaOH or 1N HCl. After cooling to 30°C, the substrate was inoculated with either *tapai* yeast or baker's yeast at varying concentrations (5%, 10%, and 15% v/v). Fermentation proceeded at 30°C for 96 h (4 days) under static conditions. Following fermentation, the broth was distilled at 78–80°C to recover bioethanol. Microbial counts for both yeast strains were determined via the total plate count (TPC) method at 24 h (initial) and 96 h (final) time points. The optimal co-culture conditions (based on performance) were further applied in a single-vessel fermentation system. The process was conducted in a water bath shaker at 35°C with agitation (100rpm) for 7–8 days to assess integrated saccharification and fermentation efficiency.

Analysis Procedure

The variables observed in the bioethanol production process through this fermentation process are bioethanol concentration, final pH of the media, substrate consumption by microbes, and bioethanol production efficiency by substrate, theoretical fermentation efficiency, and bioethanol yield.

Bioethanol concentration

The bioethanol concentration was determined using GC by comparing the retention time of the sample with that of the ethanol standard. The ethanol standard was injected with a concentration of 99.8% (v/v) (Nargotra et al., 2019).

Substrate concentration

Substrate concentration was calculated against total sugar measured using the Phenol H_2SO_4 method. Total sugar was determined by making a phenol standard curve, i.e. 2mL standard glucose solution containing 0, 10, 20, 30, 40, and 60µg glucose were each put into a test tube, 1mL of 5% phenol solution was added and shaken. Then 5mL of concentrated sulfuric acid was added quickly. Leave for 10min, shake and then place in a water bath for 15min. Absorbance was measured at 490 nm. Assaying the total sugar of the samples was carried out in the same way as the preparation of the phenol standard curve, only 2mL of glucose solution was replaced with 2mL of samples (Arnata et al., 2021).

Substrate utilization efficiency

Substrate utilization efficiency is the percentage of substrate concentration (total sugar) consumed for production to the initial substrate concentration used in production. The amount of substrate consumed is the difference between the initial total sugar concentration (So) and the sugar concentration at the end (S) of the production process (Arnata et al., 2021).

$$\text{Substrate efficiency} = \frac{(So - S)}{So} X100\% \quad (1)$$

Fermentation efficiency

Fermentation efficiency is the percentage of actual ethanol concentration to theoretical ethanol concentration. Theoretical ethanol concentration is the concentration of ethanol obtained based on the following reaction equation: $C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$. Theoretically, 100% glucose is converted into 51.1% ethanol and 48.9% CO_2 (Nargotra et al., 2019).

$$\text{Efficiency fermentation} = \frac{actualbioethanolconcentration}{theoreticalbioethanolconcetration} X100\% \quad (2)$$

Yield

The yield is the percentage of ethanol volume produced from the production process to the weight of TSW flour (Olguin-Maciel et al., 2019), namely:

$$\text{Yield (\% v/w)} = \frac{volumeofbioethanol}{TSWflourweight} X100\% \quad (3)$$

Data Analysis

Data from each research variable were analyzed for variability with ANOVA, and if the treatment significantly affected the observed variables, then the Honest Differential Test (Tukey) was conducted at the ($P<0.05$) level. The results of the diversity analysis are presented in tabular form, while the relationship between the independent variables (concentration of baker's yeast, *tapai* yeast, and substrate) and the dependent variable (measured response) of the study are presented in graphical form. The selection of the best treatment was based on the treatment that gave the highest ethanol concentration with the highest substrate utilization efficiency and yield.

RESULTS & DISCUSSION

Raw Material and Microbial Consortium Characteristics

Tapioca solid waste (TSW) exhibited high carbohydrate content (84.88 ± 0.80%), primarily as starch (60.1%), with minor fractions of cellulose (10.04%) and hemicellulose (8.89%) (Arnata et al., 2021). This composition contrasts with sweet potato residue, which contains lower starch (51.94%) but higher cellulose (18.22%) (Gou et al., 2023), suggesting TSW's superior suitability for starch-based ethanol production. Importantly, the high starch fraction indicates that process performance under CBP will be governed not only by fermentative capacity but also by the rate and extent of in situ starch depolymerization and the physical accessibility of starch granules in the slurry (i.e., mass transfer and rheology constraints at higher solids) (Raj et al., 2022). The microbial consortium of baker's yeast (*Saccharomyces cerevisiae*) and *tapai* yeast (a mixed culture of amylolytic fungi/lactic acid bacteria) showed rapid growth, with cell counts increasing from 2.83×10^4 CFU/g (baker's yeast) and 4.32×10^4 CFU/g (*tapai* yeast) at 24 h to uncountable levels by 96 h. This confirmed the consortium's viability under fermentation conditions. Such rapid proliferation also implies strong substrate demand that can increase competition for available sugars and shift carbon

partitioning among ethanol, biomass and organic acids depending on consortium balance (Nenciarini et al., 2023). The number of microbes in baker's yeast and *tapai* yeast at 24 h and 96 h incubation period (after fermentation) are presented in Fig. 1.

Bioethanol Production

The interaction between baker's yeast, *tapai* yeast, and substrate concentrations significantly influenced bioethanol production ($P<0.05$). Although the highest ethanol titer in Table 1 was observed at 15% *tapai* yeast, 10% baker's yeast, and 5% substrate (86.63g/L), the treatment 10% baker's yeast, 10% *tapai* yeast, and 10% substrate was selected as the optimal condition because it provided a balanced performance across ethanol concentration, substrate consumption, fermentation efficiency, and yield (i.e., not solely maximizing titer). This distinction is critical for scale-up, where overall yield and process robustness typically outweigh peak flask-level titers, while lower (5%) or higher (15%) concentrations resulted in reduced yields.

Table 1: Bioethanol concentration (g/L) in the treatment variation of *tapai* yeast concentration, baker's yeast, and substrate

Tapai yeast (v/v)	Substrate (w/v)	Baker's yeast (v/v)		
		5%	10%	15%
5%	5%	37.13^{efghi}	52.22^{cdefgh}	42.16^{defghi}
	10%	22.88^{i}	35.24^{fghi}	31.52^{fghi}
	15%	33.02^{fghi}	25.15^{i}	31.03^{ghi}
10%	5%	61.48^{bcde}	81.40^{ab}	63.86^{abcd}
	10%	67.71^{abc}	71.65^{abc}	55.62^{cdef}
	15%	28.97^{hi}	33.43^{fghi}	35.04^{fghi}
15%	5%	72.74^{abc}	86.63^{a}	72.74^{abc}
	10%	50.13^{cdefgh}	54.63^{cdefg}	38.34^{efghi}
	15%	31.11^{ghi}	24.35^{i}	30.39^{ghi}

Note: Mean values with different superscripts are significantly different ($P<0.05$).

As shown in Fig. 2, the highest bioethanol concentration was achieved using 10% substrate concentration with both baker's yeast and *tapai* yeast. Increasing the inoculum concentration from 5% to 10% enhanced bioethanol production by 75.47% for *tapai* yeast, whereas baker's yeast showed only a 12.42% increase. Mechanistically, this suggests that the CBP bottleneck at low *tapai* inoculum is likely upstream (hydrolysis/saccharification), where insufficient amylolytic activity limits glucose release, whereas baker's yeast primarily affects downstream glucose-to-ethanol conversion once fermentable sugars are available (Singhania et al., 2022). If use low substrate concentrations at 5%, resulted in reduced total sugar availability, limiting microbial glucose uptake and subsequent bioethanol conversion. Conversely, higher substrate concentrations led to elevated medium viscosity, causing in homogeneous yeast distribution and lower bioethanol yields (Mardawati et al., 2019). At higher solids, increased viscosity reduces effective mixing and diffusion, which can (i) reduce enzyme–substrate contact, (ii) constrain hydrolysis kinetics, and (iii) create microenvironments with local sugar depletion and inhibitory metabolite accumulation, ultimately lowering apparent fermentation efficiency even when viable cells are present (Raj et al., 2022). Similar studies was conducted previously, however bioethanol concentrations obtained in this study via the consolidated bioprocessing (CBP) method differ from previously reported values for example corn starch with *Saccharomyces cerevisiae* Y294 produced 89.35–98.13g/L (Cripwell et al., 2019), cassava starch with *Kluyveromyces marxianus* YRL 009: 79.75g/L (Wang et al., 2014), and corn starch with recombinant *Saccharomyces cerevisiae* (expressing glucoamylase/α-amylase), 80.90g/L (Kim et al., 2011) and finally potato starch with engineered *Bacillus subtilis* produced only 16.3–21.5g/L (Maleki et al., 2021). Brewer's spent grains with *Aspergillus oryzae* and *Saccharomyces cerevisiae* NCYC479 37g/L (Wilkinson et al., 2017), while sorghum straw with *Candida* sp., 30.32–38.12g/L (Adelabu et al., 2018). The comparison of production methods and bioethanol characteristics on different types of raw materials can be seen in Table 2. Therefore, differences versus prior CBP reports can be rationalized by substrate chemistry (starch vs lignocellulose), solids loading (rheology), and whether engineered enzyme expression or added enzymes were used factors repeatedly highlighted as key determinants of CBP productivity and scalability (Singhania et al., 2022).

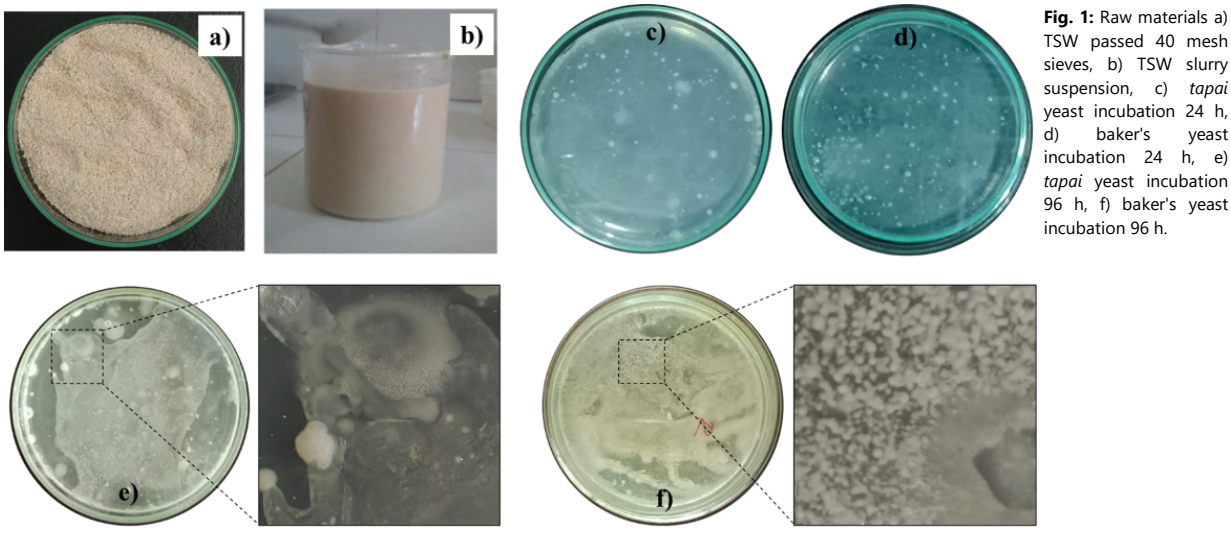

Fig. 1: Raw materials a) TSW passed 40 mesh sieves, b) TSW slurry suspension, c) *tapai* yeast incubation 24 h, d) baker's yeast incubation 24 h, e) *tapai* yeast incubation 96 h, f) baker's yeast incubation 96 h.

Table 2: Comparison of production methods and bioethanol characteristics on different types of raw materials

Methods	Raw materials	Microorganism /Processes condition	Characteristics	References
Consolidated bioprocessing	Sweet potato residue	*S. cerevisiae*, engineered	Ethanol concentration: 34.8g/L and 20.9% yield	(Wang et al., 2024)
Consolidated bioprocessing	Brewers spent grains	*Fungal consortia* (*Aspergillus niger* B2484 and *Trichoderma asperellum* B1581 strains)	Ethanol concentration: 37g/L (10 days), lower productivity	(Ghazali et al., 2021)
Consolidated bioprocessing	Agave juice	*Kluyveromyces marxianus*	Ethanol concentration: 22g/L and 99% yield at 40 ºC	(Hern´andez-Mendoza et al., 2024)
Consolidated bioprocessing	Rice straw, cellulosics	*Clostridium* sp.	Up to 2-fold yield improvement (co-culture); 83% theoretical yield	(Liu et al., 2020)
Consolidated bioprocessing	Raw corn starch	*Saccharomyces cerevisiae* Y294, substrate 200g/L, 30 ºC, 192 h	EtOH concentration: 89.35–98.13g/L., efficiency fermentation 80%	(Cripwell et al., 2019)
Consolidated bioprocess	Raw flour of *Brosimum alicastrum* seeds	*Rametes hirsuta* Bm-2, substrate:14% w/v, pH 5–5.5, temperature 32 ºC, 12 days	EtOH concentration: 13g/L, Yield: 0.97%	(Olguin-Maciel et al., 2019)
Consolidated bioprocess	Raw starch	*Saccharomyces cerevisiae* L20 dT8, substrate: 2% w/v, temperature 50 ºC, 6 days	EtOH concentration: 0.67g/L, 6% of theoretical yield	(Gronchi et al., 2022)
Consolidated bioprocessing	Starch	*Scheffersomyces shehatae*. 10% starch, 25°C, 6–10 days	EtOH concentration reached 9.21g/L with rate of ethanol production (0.92g/L/d after 10 days)	(Tanimura et al., 2015)
Consolidated bioprocessing	Potato starch	Metabolically engineered *Bacillus subtilis* strains. Substrate 50–150g/L, temperature 37°C pH 7, 96 h	EtOH concentration 16.3–21.5g/L for 96 h	(Maleki et al., 2021)
Consolidated bioprocessing	Raw starch	*S. cerevisiae* strains expressing α-amylases and glucoamylases, substrate: 200g/L, temperature 30°C, 6 days	EtOH concentration: 45.77g/L, 43.90% theoretical yield	(Sakwa et al., 2018)
Consolidated bioprocessing	Lignocellulose brewers spent grains	*Aspergillus oryzae* and *Saccharomyces cerevisiae* NCYC479, substrate 250g/L, temperature 30°C, 10 days	EtOH concentration: 37g/L, Yield 9.4%	(Wilkinson et al., 2017)
Consolidated bioprocessing	Lignocellulose-*Parthenium hysterophorus*	*S. cerevisiae* NCIM 3078 and *Pichia stipitis* NCIM 3497, substrate 213.89g/kg, pH medium 5.6, temperature 50°C, 3 days	EtOH concentration: Yield 8.15%, 23% theoretical yield	(Nargotra et al., 2019)
Direct conversion	Potato starch	*Candida albicans*, substrate concentration 5% w/v, pH 4.6 temperature 35°C for 2 days	Yield 43.70% w/w	(Aruna et al., 2015)
Single step process	Lignocellulose sorghum straw	*Candida* sp., substrate 2.5–15% w/v, pH 4.0–7.0, temperature: 30–60°C, for 3 days	EtOH concentration: 30.32–38.12g/L, fermentation efficiency 39–42%	(Adelabu et al., 2018)
Consolidated bioprocessing	Tapioca Solid Waste (TSW)	*Ragi* yeast and baker's yeast, substrate 5-15% (w/v), pH 5, temperature: 30°C for 4 days.	EtOH concentration: 37.15g/L, fermentation efficiency 69.32%	In this study

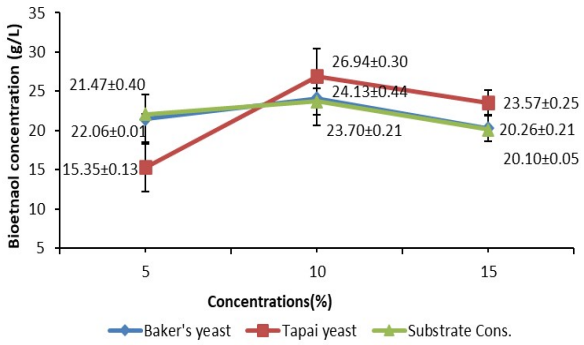

Fig. 2: Trend of correlation between concentration of baker's yeast, *tapai* and substrate concentration to bioethanol concentration.

Final pH of Fermentation Medium

The interaction between baker's yeast, *tapai* yeast, and substrate concentrations did not significantly affect the final pH of the fermentation medium ($p > 0.05$). However, the fermentation process generally reduced the initial pH (5.00) to a range of 4.73–3.74 (Table 3). This decrease is likely due to the accumulation of organic acids (e.g., acetic, lactic, and pyruvic acids) produced during fermentation. In mixed yeast-*Lactobacilli* systems, acidification is often driven by *Lactobacilli* associated lactate production and by yeast overflow metabolism/pyruvate shunting under fluctuating sugar availability, which can intensify when mass transfer is limited at higher slurry viscosities (Nenciarini et al., 2023). Pyruvic acid, formed during glycolysis, contributes to pH reduction as well. Specifically, one mole of glucose yields two moles of pyruvic acid and two moles of H^+ ions, which acidify the medium (Gunam et al., 2023). The pH trends where using *tapai* yeast, the final pH decreased with increasing baker's yeast and substrate concentrations. In baker's yeast, the final pH decreased when *tapai* yeast and substrate concentrations increased from 5% to 10%, but higher concentrations (>10%) led to a slight pH increase (Fig. 3). The slight pH rebound at higher inoculum/substrate levels is consistent with partial sugar limitation and reduced acid productivity once fermentable sugars become diffusion-limited, potentially accompanied by assimilation of some organic acids during late fermentation. This suggests that *tapai* yeast retains starch-hydrolysing activity even at elevated substrate concentrations, supporting continued bioethanol production. Similar pH declines (6.85 → 4.00) were reported in PYG (peptone-yeast-glucose) media fermented with *tapai* yeast (Gunam et al., 2023).

Table 3: Final pH value of bioethanol fermentation media in variations of concentration treatment of *tapai* yeast, baker's yeast, and substrate

Tapai yeast (v/v)	Substrate (w/v)	Baker's yeast (v/v)		
		5%	10%	15%
5%	5%	4.36^a	4.31^a	4.57^a
	10%	4.52^a	4.43^a	4.42^a
	15%	4.44^a	4.13^a	4.43^a
10%	5%	3.95^a	4.02^a	4.52^a
	10%	4.54^a	4.54^a	3.89^a
	15%	4.60^a	4.14^a	4.34^a
15%	5%	3.74^a	4.39^a	3.99^a
	10%	3.90^a	3.95^a	4.73^a
	15%	4.61^a	4.54^a	4.55^a

Noted: Initial pH: 5.0. Mean values with different superscripts are significantly different ($P<0.05$)

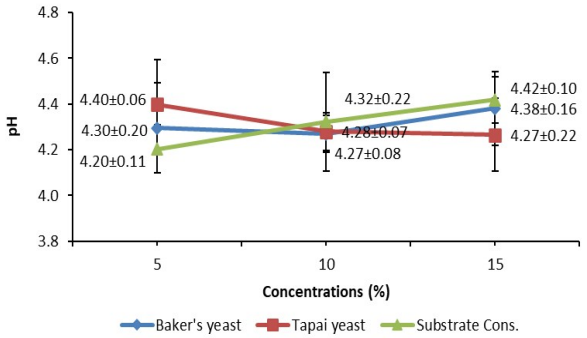

Fig. 3: Changes and relationship of pH, total sugar, and bioethanol concentration during fermentation.

Substrate Consumption by Microbes

The concentrations of baker's yeast, *tapai* yeast, and substrate showed significant interactive effects on microbial substrate consumption ($P<0.05$). When substrate concentration was maintained at 5%, all treatment combinations achieved high utilization rates ranging from 78.23% to 95.63% (Table 4). However, as revealed in Fig. 4, increasing substrate concentration led to a progressive decline in consumption efficiency. This trend was particularly evident in the reduction from 79.82% utilization at 5% substrate to just 44.08% at 15% substrate - a substantial 35.74% decrease. This pattern is consistent with "high-solids" effects where reduced free water and increased viscosity limit enzyme mobility and microbial access to hydrolysed sugars, thereby lowering the fraction of total carbohydrate that becomes biologically available within the fermentation time window (Raj et al., 2022).

Table 4: Substrate consumption by microbes (%) during bioethanol fermentation on variation of concentration treatment of *tapai* yeast, baker's yeast, and substrate

Tapai yeast (v/v)	Substrate (w/v)	Baker's yeast (v/v)		
		5%	10%	15%
5%	5%	67.21^{bcdef}	69.85^{bcdef}	83.88^{ab}
	10%	55.83^{fg}	55.56^{fg}	59.24^{defg}
	15%	44.41^{g}	50.76^{fg}	42.14^{g}
10%	5%	95.63^{a}	80.87^{abcd}	81.79^{abc}
	10%	60.56^{cdefg}	59.93^{cdefg}	51.73^{fg}
	15%	54.49^{fg}	57.19^{efg}	50.09^{fg}
15%	5%	78.23^{abcde}	79.87^{abcd}	81.06^{abcd}
	10%	56.47^{efg}	62.52^{bcdef}g	54.37^{fg}
	15%	54.46^{fg}	57.16^{efg}	50.94^{fg}

Note: Mean values with different superscripts are significantly different ($P<0.05$).

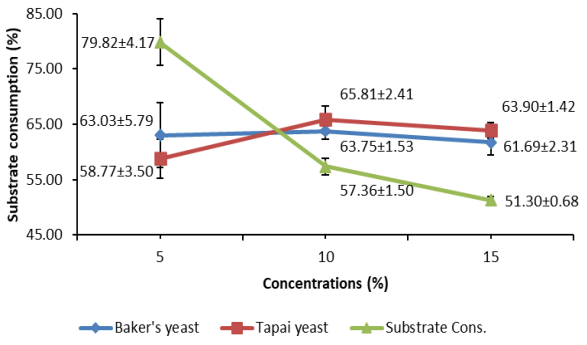

Fig. 4: Trend of the relationship between the effect of concentration of baker's yeast, *tapai* yeast, and substrate concentration on substrate consumption by microbes.

This concentration-dependent pattern appears directly related to changes in medium viscosity. Higher substrate concentrations created a more viscous environment that likely hindered starch hydrolysis by both yeast strains, ultimately restricting glucose availability (Voronovsky et al., 2009; Wijaya et al., 2025). In CBP, this rheology–hydrolysis coupling is especially important because incomplete saccharification reduces not only ethanol formation but also apparent "substrate consumption," since a portion of carbohydrate remains physically inaccessible rather than metabolically unused (Singhania et al., 2022). Conversely, the lower viscosity conditions at 5% substrate concentration provided two key advantages: (1) improved mixing homogeneity that enhanced microbial access to starch particles, and (2) more efficient enzymatic conversion of starch to glucose, thereby facilitating optimal ethanol production. From an industrial perspective, these findings support strategies such as fed-batch solids addition, staged hydrolysis-then-fermentation, or impeller/agitator optimization to reduce viscosity early and improve mass transfer at scale.

Substrate-to-Bioethanol Conversion Efficiency

The interaction between baker's yeast, *tapai* yeast, and substrate concentrations significantly influenced substrate-to-bioethanol conversion efficiency ($P<0.05$). Efficiency generally decreased with increasing substrate concentration (Table 5). High efficiency treatments (63.86–86.63%) can be seen when 10% *tapai* yeast + 10–15% baker's yeast was used at 5% substrate concentration and also at 15% *tapai* yeast + 5–15% baker's yeast at 5% substrate concentration (no significant differences between these combinations). In contrast low-efficiency treatments (22.88–42.16%) happen when use 5% *tapai* yeast + 5–15% baker's yeast at 5% substrate concentration.

Table 5: Bioethanol production efficiency by substrate (%) during bioethanol fermentation at various concentrations of *tapai* yeast, baker's yeast, and substrate

Tapai yeast (v/v)	Substrate (w/v)	Baker's yeast (v/v)		
		5%	10%	15%
5%	5%	37.13^{efghi}	52.22^{cdefgh}	42.16^{defghi}
	10%	22.88^{i}	35.24^{fghi}	31.52^{fghi}
	15%	33.02^{fghi}	25.15^{i}	31.03^{ghi}
10%	5%	61.48^{bcde}	81.40^{ab}	63.86^{abcd}
	10%	67.71^{abc}	71.65^{abc}	55.62^{cdef}
	15%	28.97^{hi}	33.43^{fghi}	35.04^{fghi}
15%	5%	72.74^{abc}	86.63^{a}	72.74^{abc}
	10%	50.13^{cdefgh}	54.63^{cdefg}	38.34^{efghi}
	15%	31.11^{ghi}	24.35^{i}	30.39^{ghi}

Note: Mean values with different superscripts are significantly different ($p<0.05$).

A conversion efficiency of 22.88% indicates only 22.88% of consumed substrate was converted to bioethanol, while 77.22% was diverted to for cellular maintenance, biomass formation and organic acid synthesis (e.g., acetic acid, lactate, pyruvate) via incomplete ethanol conversion or alternative pathways (Ma et al., 2021; Mellicha et al., 2021; Wang et al., 2021). In mixed cultures, this carbon diversion can also reflect consortium interactions (competition and cross-feeding), where rapid sugar uptake supports biomass expansion and acid formation by non-ethanol producers, reducing the fraction of carbon reaching ethanol (Nenciarini et al., 2023). In Fig. 5, peak efficiency occurred

at 10% yeast concentration (*tapai*: 55.46%; baker's: 51.63%), decline beyond 10% concentration. The efficiency dropped from 63.37% (5% substrate) to 30.27% (15% substrate), likely due to (i) increased medium viscosity impairing starch hydrolysis, and (ii) metabolic shifts toward non-ethanol products at higher concentrations.

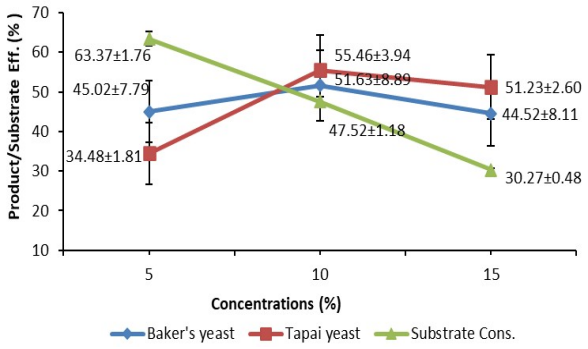

Fig. 5: Correlation trend between the effect of concentration of baker's yeast, *tapai* yeast, and substrate concentration on bioethanol production efficiency by substrate.

Theoretical Fermentation Efficiency

The concentrations of *tapai* yeast, baker's yeast, and substrate showed significant interactive effects on fermentation efficiency ($P<0.05$). The highest efficiency of 80.63% (representing 80.63% of theoretical maximum yield) was achieved with 5% *tapai* yeast + 10% baker's yeast + 5% substrate - statistically comparable to treatments using 5% concentrations of all components. In contrast, the combination of 15% *tapai* yeast + 10% baker's yeast + 15% substrate yielded much lower efficiency (37.59%). Substrate concentration played a key role in these efficiency patterns (Fig. 6, Table 6). For baker's yeast, efficiency progressively declined from 68.03% at 5% substrate to 46.75% at 15% substrate. *Tapai* yeast showed a different response, peaking at 63.05% efficiency with 10% concentration (versus 54.89% at 5% and 55.69% at 15%). Mechanistically, reduced theoretical efficiency at higher solids is consistent with incomplete carbohydrate accessibility and increased non-ethanol carbon sinks (biomass and acids), which are amplified when mass transfer limits hydrolysis and creates stress conditions that redirect metabolism away from ethanol (Raj et al., 2022). These patterns suggest that higher efficiency correlates with optimal conditions for bioethanol production whileminimizing substrate consumption.

Comparative analysis reveals that TSW fermentation efficiency via CBP differs from published values for other feedstocks. Lignocellulosic materials showed efficiencies of 39–42% for sorghum straw (Adelabu et al., 2018), 23% for *Parthenium hysterophorus* (Nargotra et al., 2019) and 41.39% for pine needles (Vaid et al., 2017). Starch-based substrates demonstrated higher efficiencies overall: 80% (Cripwell et al., 2019) for corn starch, 78.30% for cassava starch (Wang et al., 2014), 43.90% for raw starch (Sakwa et al., 2018), and 6% for crude starch (Gronchi et al., 2022). Such variation is expected because CBP performance is strongly governed by the degree of integrated saccharification–fermentation coupling, microbial robustness, and process hydrodynamics factors repeatedly identified as major barriers in CBP scale-up and commercialization (Singhania et al., 2022). These variations highlight how both feedstock type and processing conditions influence fermentation outcomes.

Table 6: Theoretical fermentation efficiency (%) during bioethanol fermentation on variation of concentration treatment of *tapai* yeast, baker's yeast, and substrate

Tapai yeast (v/v)	Substrate (w/v)	Baker's yeast (v/v)		
		5%	10%	15%
5%	5%	57.33^{abcde}	80.63^{a}	65.09^{abcde}
	10%	50.18^{abcde}	54.41^{abcde}	48.67^{bcde}
	15%	50.98^{abcde}	38.83^{de}	47.90^{bcde}
10%	5%	69.09^{abcd}	77.49^{ab}	68.61^{abcde}
	10%	66.00^{abcde}	69.32^{abcd}	66.47^{abcde}
	15%	44.72^{cde}	51.61^{abcde}	54.10^{abcde}
15%	5%	77.11^{ab}	57.21^{abcde}	59.72^{abcde}
	10%	72.33^{abc}	45.46^{cde}	56.80^{abcde}
	15%	48.04^{bcde}	37.59^{e}	46.93^{bcde}

Note: Mean values with different superscripts are significantly different ($P<0.05$).

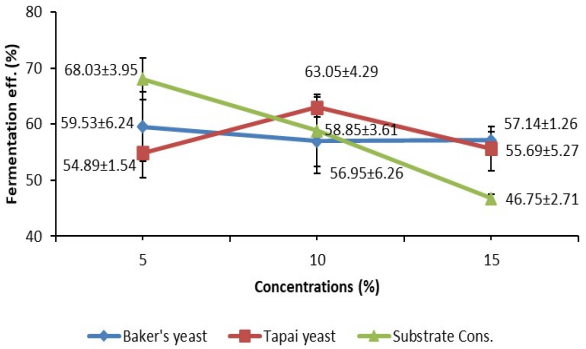

Fig. 6: Correlation trend between concentration of baker's yeast, *tapai* yeast, and substrate concentration on theoretical fermentation efficiency.

Bioethanol Yield

The experimental results demonstrated significant effects of baker's yeast, *tapai* yeast, and substrate concentrations on bioethanol yield ($P<0.05$, Table 7). Optimal production yields of 3.96-4.16% were achieved when using balanced 10% concentrations of all components, including baker's yeast, *tapai* yeast, and substrate. In contrast, two suboptimal regimes were identified. The first showed lower yields of 1.19–2.23% with 5% *tapai* yeast combined with 5–15% baker's yeast and substrate, while the second demonstrated moderately improved but still suboptimal yields of 2.04–3.47% with 15% *tapai* yeast, 15% substrate, and 5–15% baker's yeast.

Table 7: Bioethanol yield in various treatments of *tapai* yeast concentration, baker's yeast concentration and substrate

Tapai yeast (v/v)	Substrate (w/v)	Baker's yeast (v/v)		
		5%	10%	15%
5%	5%	1.19^{g}	1.77^{fg}	1.74^{fg}
	10%	1.23^{g}	1.87^{efg}	1.82^{fg}
	15%	2.21^{cdefg}	1.85^{efg}	1.90^{efg}
10%	5%	2.91^{abcdef}	3.19^{abcde}	2.53^{cdefg}
	10%	3.96^{ab}	4.16^{a}	2.78^{bcdef}
	15%	2.30^{cdefg}	2.77^{bcdef}	2.57^{cdefg}
15%	5%	2.76^{bcdef}	3.42^{abc}	2.85^{abcdef}
	10%	2.72^{bcdef}	3.30^{abcd}	1.98^{cdefg}
	15%	2.47^{cdefg}	2.04^{defg}	2.25^{cdefg}

Note: Mean values with different superscripts are significantly different ($P<0.05$).

Analysis of the concentration-dependent relationships (Fig. 7) revealed several key patterns. For both yeast types, the 10% concentration consistently produced superior results compared to either 5% or 15% treatments. Substrate concentration showed an inverse relationship with yield, primarily due to elevated medium viscosity impairing homogeneous yeast distribution, compromised starch hydrolysis efficiency reducing glucose availability, and subsequent depression of ethanol production potential. This viscosity-driven yield penalty is directly relevant for industrial translation, because high-solids fermentation is commonly targeted to increase volumetric productivity, yet it can backfire when mixing/heat transfer limits reduce effective conversion (Raj et al., 2022).

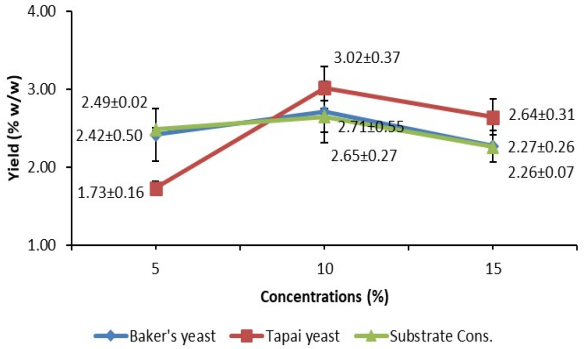

Fig. 7: Trend of correlation between concentration of baker's yeast, *tapai* yeast, and substrate concentration on bioethanol yields.

Comparative analysis with published studies revealed distinct yield patterns based on feedstock type. Lignocellulosic materials generally produced lower yields, including *Brosimum alicastrum* seeds at 0.97% (Olguin-Maciel et al., 2019), pine needle at 0.148% (Vaid et al., 2017; Olguin-Maciel et al., 2019), brewer's spent grains at 9.4% (Wilkinson et al., 2017), *Parthenium hysterophorus* at 8.15% (Nargotra et al., 2019) and wheat bran at 18% (Zerva et al., 2014). In contrast, starchy materials showed significantly higher conversion efficiencies, with corn starch at 80% (Sakwa et al., 2018), cassava at 40% (Wang et al., 2014), and potato at 43.70% (Aruna et al., 2015).

This yield disparity fundamentally stems from the structural differences between feedstocks. The more accessible α-glyosidic bonds in starch facilitate enzymatic hydrolysis to fermentable glucose, while the complex lignin-hemicellulose matrix of lignocellulosic materials creates greater recalcitrance to biological degradation. The results clearly demonstrate how both microbial concentrations and substrate characteristics critically influence bioethanol production efficiency.

Fermentation in Bioethanol Production and Substrate Utilization

Fig. 8 illustrates the dynamic changes in bioethanol concentration, total sugar content, and media pH throughout the fermentation process. A progressive decrease in substrate concentration (total sugar) from an initial 109.80g/L to 35.43g/L was observed during the first three days of fermentation, after which sugar levels stabilized. This reduction correlated with a gradual pH decline from 5.0 to 4.4 over the same period. The inverse relationship between these parameters and bioethanol production became evident as the ethanol concentration rose to 36.37g/L during this three-day phase. This trajectory supports a two-phase CBP interpretation: an early phase dominated by active saccharification and rapid fermentation (high sugar flux), followed by a late phase where acidification and reduced sugar release (due to depleted accessible starch and/or viscosity-limited hydrolysis) constrain further ethanol accumulation (Afedzi & Parakulsuksatid, 2023).

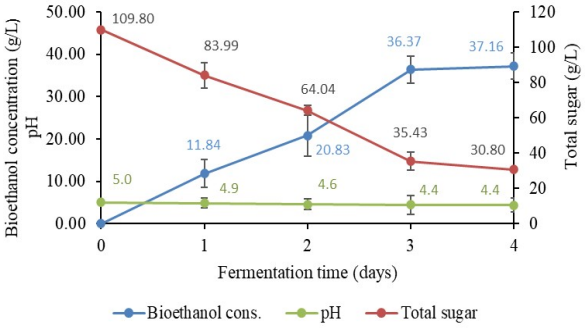

Fig. 8: Changes and relationship of pH, total sugar, and bioethanol concentration during fermentation.

An extending fermentation to four days did not yield significant additional ethanol production. This plateau likely results from the increasingly acidic medium conditions, as numerous studies have documented how pH values below 4.5 can substantially inhibit microbial activity and fermentation performance (Mohseni et al., 2016). These observed patterns align with previous research findings. Similar dynamics were reported in cassava starch fermentation using *Kluyveromyces marxianus* YRL 009, where total sugar decreased from 200g/L to 30.55g/L while bioethanol increased from 0g/L to 72.99g/L over eight days (Wang et al., 2014). Comparable trends were also observed in sweet potato residue fermentation with *Saccharomyces cerevisiae* (Gou et al., 2023), suggesting these relationships represent fundamental aspects of ethanol fermentation across different substrate-microorganism systems.

Conclusion and Prospects for Sustainable Bioethanol Production

This study establishes, for the first time, the technical feasibility of using a non-GMO microbial consortium of baker's and *tapai* yeast for direct bioethanol production from tapioca solid waste (TSW) through consolidated bioprocessing (CBP). The optimal production system, employing balanced 10% concentrations of both yeasts and substrate, delivered exceptional performance across multiple metrics: bioethanol concentrations reaching 20.51–37.15g/L, substrate conversion efficiency of 59.93%, production efficiency of 71.65%, theoretical fermentation efficiency of 69.32%, and an overall yield of 4.16% w/w. While these biological and process parameters showed

significant concentration-dependent effects, the final media pH remained relatively stable within the 4.73–3.74 range. The CBP methodology demonstrates clear advantages over conventional sequential hydrolysis and fermentation approaches, particularly in terms of economic viability through its simplified single-step operation. For industrial scale-up, the principal technical challenge highlighted by this work is the viscosity–mass transfer constraint at higher solids, implying that reactor hydrodynamics (mixing power, impeller selection, and feeding strategy) must be engineered to preserve effective saccharification–fermentation coupling. Scaling this technology could yield substantial benefits for Indonesia's energy sector, where the annual 4–6 million tons of TSW by-product could generate 166,503–249,754kg of bioethanol – a meaningful offset to fossil fuel consumption. From a sustainability perspective, recent cassava-based life cycle assessment (LCA) studies indicate that integrating residue valorisation and energy recovery (e.g., stillage-to-biogas/combined heat and power (CHP) can substantially improve greenhouse-gas performance and overall resource efficiency, supporting the role of cassava-waste CBP within circular bioeconomy systems. Environmental analyses further support this approach, with comparable cassava-based systems demonstrating greenhouse gas reductions of 26–39kg CO_2eq/GJ according to life cycle assessment studies. Several challenges must be addressed to realize this potential. The process requires further optimization of critical parameters including pH control, temperature regulation, agitation dynamics, and trace element supplementation. Downstream processing faces technical hurdles due to the azeotropic nature of ethanol-water mixtures, while environmental trade-offs between reduced greenhouse emissions and potential increases in acidification/eutrophication from agricultural inputs need careful management (Jeswani et al., 2025).

Future research should focus on comprehensive life cycle assessments specific to TSW-derived bioethanol, coupled with techno-economic analyses of full-scale implementation. In particular, scale-down/computational fluid dynamics (CFD)-informed approaches are recommended to anticipate mixing-driven performance losses and to design control strategies that maintain consortium stability and productivity in large reactors. Parallel development of energy-efficient separation technologies and integration with circular economy principles could maximize both environmental benefits and socioeconomic returns through job creation and complete waste valorisation. This work positions CBP with native yeast consortia as a technologically sound and sustainable solution for TSW conversion, combining process efficiency with environmental benefits. With continued development, this approach could transform Indonesia's cassava industry into a key contributor to renewable energy solutions while addressing critical waste management challenges. The demonstrated system offers a practical foundation for sustainable biofuel production that merits further investigation and scale-up efforts.

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