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Optimization of IoT-Based Molasses Fermentation Process Using Saccharomyces Cerevisiae For Enhanced Bioethanol Yield

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Abstract
Bioethanol fermentation from molasses is one strategy for turning sugar-industry waste into a renewable energy source. This study designed and built an Internet of Things (IoT)-based bioethanol fermentation system integrated with temperature (DS18B20), pH (PH-4502C), CO_2 gas (MQ-135), ethanol vapor (MQ-3), pressure (HX710B), and bubble-counter (FC-03) sensors on an ESP32 module, equipped with a real-time monitoring dashboard and data-logging system. Fermentation was carried out using Saccharomyces cerevisiae at molasses concentrations of 15, 16, and 18 °Brix, with 2 g/L ammonium-sulfate (ZA) nutrient supplementation, an operating temperature of 32–36 °C, and a 48-hour fermentation time. The actual bioethanol yield reached 20.94% (efficiency 40.98%) at 16 °Brix, 20.24% (39.60%) at 15 °Brix, and 15.27% (29.89%) at 18 °Brix, even though 18 °Brix recorded the highest sugar conversion (54.80%); this discrepancy is attributed to carbon diversion toward the High-Osmolarity-Glycerol (HOG) pathway under elevated osmotic pressure. The CO_2-proxy and ethanol-estimate profiles showed qualitatively consistent trends that aligned with the early fermentation rate across variations, but were non-selective and prone to saturation, so they are valid only as trend indicators rather than absolute measurements. The optimum molasses concentration was found at 16 °Brix, providing a basis for refining IoT-based bioethanol fermentation monitoring in future work.
Keywords: *bioethanol, fermentation, internet of things (iot), molasses, saccharomyces cerevisiae.*

Abstrak
Fermentasi bioetanol berbahan baku molase merupakan salah satu pendekatan pemanfaatan limbah industri gula sebagai sumber energi terbarukan. Penelitian ini merancang dan merealisasikan sistem fermentasi bioetanol berbasis Internet of Things (IoT) yang terintegrasi dengan sensor suhu (DS18B20), pH (PH-4502C), gas CO_2 (MQ-135), etanol (MQ-3), tekanan (HX710B), dan penghitung gelembung (FC-03) pada modul ESP32 yang dilengkapi dashboard monitoring dan data logging secara real-time. Fermentasi dilakukan menggunakan *Saccharomyces cerevisiae* pada variasi konsentrasi molase 15, 16, dan 18 °Brix dengan suplementasi nutrisi ZA 2 g/L, suhu operasi 32–36 °C, dan durasi fermentasi 48 jam. Hasil penelitian menunjukkan yield bioetanol aktual pada Brix 16% (20,94%; efisiensi 40,98%), Brix 15% (20,24%; efisiensi 39,60%), dan Brix 18% (15,27%; 29,89%), meskipun Brix 18% mencatat konversi gula tertinggi (54,80%), yang diduga terjadi akibat pengalihan karbon menuju jalur High Osmolarity Glycerol (HOG) akibat tekanan osmotik tinggi. Profil CO_2 proxy dan estimasi etanol menunjukkan pola kualitatif yang konsisten serta selaras dengan laju awal fermentasi antarvariasi, namun bersifat non-selektif dan mengalami saturasi sehingga hanya sahih digunakan sebagai indikator tren. Konsentrasi molase optimum berada pada Brix 16% dan memberikan dasar evaluasi untuk penyempurnaan sistem monitoring fermentasi bioetanol berbasis IoT pada penelitian selanjutnya.
Kata Kunci: *bioetanol, fermentasi, internet of things (iot), molase, saccharomyces cerevisiae*

1. Introduction

Growing energy demand is driving wider use of renewable energy to replace fossil fuels. Bioethanol is a biofuel produced by fermenting sugar-based biomass and is used as a renewable-energy component [1]. Molasses, a byproduct of the sugar industry, still contains fermentable sugar reported in the range of 50–65% [2], chemically dominated by sucrose (about 35%), glucose (7%), and fructose (9%) [3], sugars that S. cerevisiae metabolizes readily through glycolysis, making molasses usable directly as a fermentation substrate without complex hydrolysis.

Bioethanol fermentation converts sugar into ethanol and carbon dioxide (CO_2) through the glycolytic pathway [4]. Because ethanol formation is stoichiometrically linked to CO_2 release, the CO_2 concentration profile over time can be used to describe fermentation activity [5]. Molasses fermentation typically uses S. cerevisiae operating at 30–34 °C and pH 4.0–5.0 [5]. Maintaining these operating conditions is important to support yeast metabolic activity and promote efficient conversion of fermentable sugars into ethanol.

Conventional fermentation monitoring through periodic liquid sampling produces discrete data that poorly represents a continuous process [5]. Internet of Things (IoT) technology the integration of physical devices with computing and network systems for real-time data acquisition and transmission [6] offers an alternative. Gas-based monitoring using CO_2 has been used as an indirect fermentation indicator [7], while ethanol is also detectable in the gas phase owing to vapor–liquid equilibrium; integrating CO_2 and ethanol gas sensors with an IoT system enables more representative, real-time fermentation monitoring [8]. Such an approach can provide continuous process information, allowing changes in fermentation conditions and gas production to be observed without relying solely on periodic sampling.

Several prior studies on molasses-to-bioethanol fermentation have focused on process parameters. Rachmanto & Sutanto [1], Egamiati et al. [9], and Nurchayati et al. [2] examined the effect of pH, °Brix, and yeast concentration, showing that fermentation conditions affect bioethanol output, although monitoring still relied on periodic liquid sampling; Septiawan et al. [5] and Rachmanto et al. [4] examined the effect of fermentation time and temperature on bioethanol yield. Other studies have applied IoT to fermentation monitoring: Baicu et al. [10], Firmansyah et al. [11], and Jatmiko & Buwono [12] showed that IoT can provide real-time process data, and similar IoT-based pH and temperature monitoring has also been demonstrated in coffee fermentation [13], but this body of work remained limited to liquid-phase parameters. Gas sensors as a fermentation indicator have been demonstrated by Sani & Dahlan [14] and Cahyandari & Harmadi [15], but had not yet been applied to molasses fermentation for bioethanol-yield evaluation.

Building on this gap, the present study integrates molasses fermentation with an IoT monitoring system based on a CO_2 gas sensor to evaluate the fermentation rate and the consistency of bioethanol yield. The specific objectives are to (1) design and realize an IoT-based molasses fermentation system integrated with a CO_2 gas sensor for monitoring at 32–36 °C; (2) analyze the relationship between the CO_2 concentration profile over time and bioethanol formation under controlled-temperature fermentation; (3) analyze the effect of molasses concentration variation (15, 16, and 18 °Brix) on bioethanol yield; and (4) evaluate the performance of the IoT-based monitoring system in maintaining consistent bioethanol yield and fermentation efficiency.

2. Material and Methods

Materials and equipment

Fermentation was carried out in a laboratory-scale acrylic fermentor (3.5 L working volume of a 5.3 L geometric capacity) equipped with a magnetic stirrer (95 rpm), a 75 W aquarium heater for temperature control, and an acrylic gas holder operating on the water-displacement principle. The DS18B20 temperature sensor and PH-4502C pH sensor were placed inside the fermentor; the MQ-135 CO_2 sensor and MQ-3 ethanol-vapor sensor metal-oxide-semiconductor (MOS) devices whose electrical resistance changes as target-gas molecules adsorb onto a heated SnO_2 sensing layer [16] were mounted at the headspace outlet, since the headspace accumulates fermentation gases and can be monitored without direct contact with the liquid phase [17]. An HX710B pressure sensor was fitted on the gas line and an FC-03 bubble-counter sensor at the gas outlet; all sensors were acquired through an ESP32 microcontroller. **Fig. 1** shows the layout of the realized system. The raw materials were Saccharomyces cerevisiae yeast, ZA fertilizer (ammonium sulfate) as a supplementary nitrogen source, sugarcane molasses obtained from PT PG Rajawali II (Jatitujuh Unit), and distilled water.

Fig. 1: IoT-based fermentation system
Source: Personal documentation

Research procedure

The overall research flow is shown in **Fig. 2**. Molasses was first characterized (°Brix, pH, density) and pretreated by decalcification with concentrated H_2SO_4 to pH 3.9, heating at 95 °C for 10 minutes, settling for 24 hours, filtering, and autoclave sterilization (121 °C, 15 minutes). Because the high solute concentration of molasses raises the osmotic pressure of the medium and can suppress microbial activity if left uncontrolled [18], the sterilized molasses was diluted with water to three substrate concentrations of 15, 16, and 18 °Brix. A liquid inoculum was prepared by dissolving Glucose Yeast Extract Broth (1.75 g yeast extract, 3.5 g tryptone/peptone, 7 g glucose in 350 mL distilled water), inoculating with 3.5 g of S. cerevisiae culture, and incubating in a shaker incubator for 24 hours.

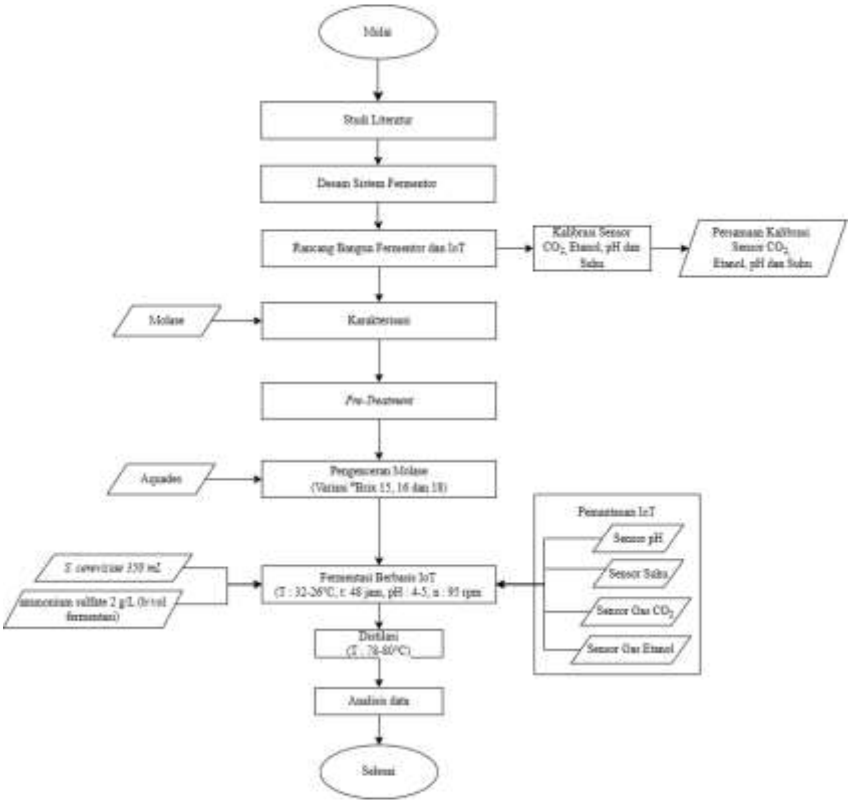

Fig. 2: Flow diagram of the research stages.

Each fermentation run combined 3150 mL of diluted molasses substrate (pH adjusted to 5), 350 mL of liquid inoculum (10% v/v), and 2 g/L ZA nutrient in the 3,5 L fermentor, stirred at 95 rpm. Because molasses is often nitrogen-limited for yeast growth, ZA supplementation was included on the strength of evidence that added nitrogen and phosphate increase yeast growth rate and ethanol output [19], including reported gains from ammonium-sulfate dosing near 4 g/L in stress-tolerant strains [20]. Fermentation proceeded batch-wise in a closed system without external aeration for 48 hours at 32–36 °C, while temperature, CO_2 gas, and ethanol vapor were logged in real time. Before use, all six sensors were calibrated or characterized: DS18B20 against a reference digital thermometer; PH-4502C using pH 4.01 and 6.86 buffers; MQ-135 against ambient air (390–420 ppm CO_2) after a 24-hour burn-in; MQ-3 against the ethanol-vapor headspace of 5–25% ethanol solutions after a 24-hour burn-in; HX710B via zero-offset and a water-column manometer; and FC-03 functionally, using a bubble chamber filled with vegetable glycerin.

At the end of fermentation, a representative aliquot was withdrawn from the mid-height of the fermentor after homogenizing the medium, following representative-sampling principles for viscous, potentially stratified media [21]. The aliquot was distilled at 78–80 °C, exploiting ethanol's lower boiling point relative to water [22], and the ethanol content of the distillate (% w/w) was determined from its density by pycnometry, with the density to ethanol content relationship obtained by interpolation on a standard ethanol–water density reference [23].

Yield and efficiency calculation

Molasses fermentation proceeds in two biochemical steps: enzymatic hydrolysis of sucrose to glucose and fructose (Eq. 1), followed by fermentation of the resulting hexoses to ethanol and carbon dioxide (Eq. 2).

$$C_{12}H_{22}O_{11} + H_2O \rightarrow 2\ C_6H_{12}O_6 \ \ (invertase) \tag{1}$$

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2 \ \ (zymase) \tag{2}$$

Initial and residual fermentable-sugar mass were estimated from a component mass balance using the measured °Brix and the density of the diluted molasses solution at t = 0 and t = 48 h (Eq. 3).

$$m\ sugar = V\ medium \times \rho\ solution \times (Brix / 100) \tag{3}$$

The actual mass of ethanol produced was back-calculated from the distillate content (Eq. 4) and scaled to the total fermentor volume using the ratio of total medium volume to aliquot volume (Eq. 5).

$$m\ ethanol,dist = \%ethanol \times \rho\ dist \times V\ dist \tag{4}$$

$$m\ EtOH,tot = m\ EtOH,dist \times (Vtot / Vsample) \tag{5}$$

The actual bioethanol yield is the ratio of the total ethanol mass produced to the initial sugar mass available as substrate [24] (Eq. 6).

$$Yield\ actual\ (\%) = (m\ EtOH,tot / m\ sugar) \times 100\% \tag{6}$$

Under ideal stoichiometry (Eq. 2), one mole of glucose (180 g/mol) yields two moles of ethanol (46 g/mol), giving a theoretical (Gay-Lussac) yield of 0.511 g ethanol per g glucose — the thermodynamic upper bound for sugar-to-ethanol conversion [24]. Fermentation efficiency expresses how closely the actual yield approaches this limit (Eq. 7).

$$Eff\ (\%) = (Yield\ actual / 0.511) \times 100\% \tag{7}$$

3. Results and Discussion

IoT-based fermentor system

The IoT-based bioethanol fermentor was successfully realized as an integrated laboratory-scale system consisting of the fermentor and gas-holder unit, an ESP32-based data-acquisition circuit wired to all six sensors, and a monitoring layer comprising a local React/FastAPI dashboard - updated over WebSocket for refresh-free, full-duplex data delivery [25] plus a Blynk-Cloud dashboard for remote access, so that fermentation could be observed continuously over the 48-hour run without manual logging.

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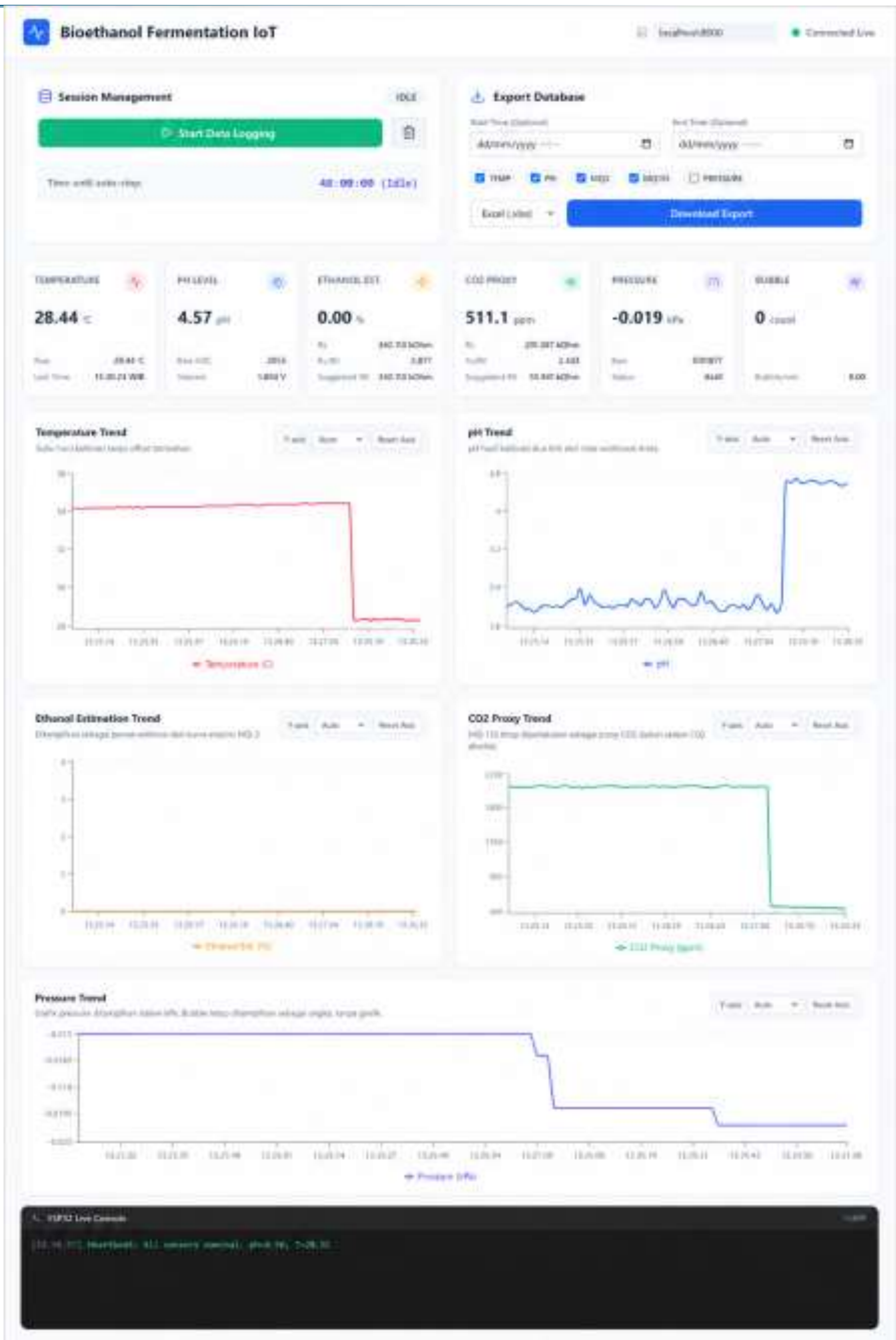

Fig. 3: React-Based Monitoring Dashboard
Source: Personal Documentation

Sensor validation

Before use in fermentation trials, all sensors were validated against reference instruments. The DS18B20 temperature sensor showed a deviation of only 0.01–0.04 °C from a reference digital thermometer, well within its ±0.5 °C manufacturer specification. The PH-4502C pH sensor produced a strongly linear voltage–pH relationship ($V = -0.167(pH) + 2.5471$, $R^2 = 0.9966$) against standard buffers, which was inverted into the calibration equation implemented on the ESP32. The MQ-135 CO_2 sensor showed a stable baseline resistance (287.1–306.1 kΩ, mean 296.99 kΩ) in ambient air but, consistent with its non-selective MOS sensing principle, was not calibrated against an absolute CO_2 standard; its output was therefore treated as a relative CO_2 proxy rather than an absolute concentration. The MQ-3 ethanol sensor was characterized against 0–25% ethanol-vapor headspace concentrations and fitted with a power-law model ($Rs = 11.771 \times [ethanol\%]^{-0.162}$, $R^2 = 0.9431$) used to estimate relative ethanol-vapor changes. The FC-03 bubble counter, tested functionally with a vegetable-glycerin bubble chamber, registered pulses correctly in isolation, but together with the HX710B pressure module a 24-bit load-cell-amplifier device commonly used for pressure and weight sensing [26] it produced no bubble or pressure data during any of the three fermentation runs, indicating that fermentation gas escaped through minor leaks elsewhere in the fermentor rather than through the intended sensor path; both are therefore considered not yet reliable for this fermentor configuration.

Fermentation monitoring profiles

Across all three molasses concentrations, the temperature sensor maintained the medium within the intended 32–36 °C band, with a repeating ~1 °C saw-tooth fluctuation characteristic of on–off thermostat control, confirming that DS18B20 combined with the heater control loop is reliable for real-time temperature monitoring. The MQ-135 CO_2-proxy signal followed a consistent rise–plateau–decline–rise pattern in all three variations, with the fastest initial rise in the 18 °Brix run, followed by 15 °Brix and then 16 °Brix. All three variations reached a similar apparent ceiling of about 2972 ppm before declining, better interpreted as sensor saturation than as the true maximum CO_2 concentration inside the fermentor, since a semiconductor gas sensor's response flattens once its surface adsorption sites approach saturation [27]. Because MQ-135 is sensitive to several gases besides CO_2, including ammonia and volatile organics [28], and because the late-fermentation rise coincided with the onset of the MQ-3 ethanol signal while the pressure sensor showed no corresponding change, the late-stage increase is interpreted as a shift in headspace gas composition rather than renewed CO_2 production. Taken together, the gas-proxy signals are best read as qualitative trend indicators of fermentation progress rather than quantitative gas-concentration measurements.

Effect of molasses concentration on yield and efficiency

Bioethanol yield is shaped by feedstock type, microorganism, and the stability of fermentation conditions [29]. **Table 1** summarizes the substrate and fermentation-performance parameters measured for the three molasses concentrations after 48 hours.

Table 1. Fermentation parameters at different molasses concentrations.

Parameter	Brix 15%		Brix 16%		Brix 18%		Unit
	t (h) = 0	**t (h) = 48**	**t (h) = 0**	**t (h) = 48**	**t (h) = 0**	**t (h) = 48**	
Brix	15	7.5	16	8.5	18	8.7	%
Fermentation pH	5	5	5.06	5	4.9	4.8	-
Density (ρ)	1.0736	1.0065	1.0759	1.0128	1.0849	1.0146	gram/mL
Sugar Mass	563.7	264.2	602.5	301.3	683.5	309.0	gram
Converted Sugar	299.5		301.2		374.5		gram
Sugar Conversion	53.13		49.99		54.80		%
Average Temperature	32.106		34.153		34.437		°C

Source: processed primary data (fermentation results).

Table 2. Distillation characteristics and fermentation performance at different molasses concentrations.

Parameter	Brix 15%	Brix 16%	Brix 18%	Unit
Distillate pH	5.6	5.9	5.9	-
Distillate Temperature	28	27	27	°C
Distillate Volume (Aliquot 100 mL)	6.1	6.2	5.8	mL
Density Distillate (ρ)	0.88329	0.86908	0.89055	gram/mL
Total Distillate Volume	213.5	217	203.0	mL
Ethanol Concentration in Distillate	60.49	66.89	57.74	% v/v
Total m_{etanol}	114.07	126.155	104.382	gram
Actual Yield	20.24	20.94	15.27	%
Fermentation Efficiency	39.60	40.98	29.89	%

Source: processed primary data (distillation results).

All three variations showed a clear decline in °Brix over 48 hours: 15 °Brix fell to 7.5% and 16 °Brix to 8.5% (both Δ = 7.5 points), while 18 °Brix fell to 8.7% (Δ = 9.3 points); sugar conversion was 53.13%, 49.99%, and 54.80%, respectively. The larger absolute Brix drop and higher sugar conversion at 18 °Brix reflect its greater initial substrate availability, consistent with a similar relative decline reported for 14–16 °Brix molasses fermentation over 48 hours [5]. Medium pH decreased only slightly in all variations (from 4.9–5.06 to 4.8–5.0), remaining within the 3.5–6.5 tolerance range of S. cerevisiae, so pH is unlikely to have limited fermentation in any of the three runs.

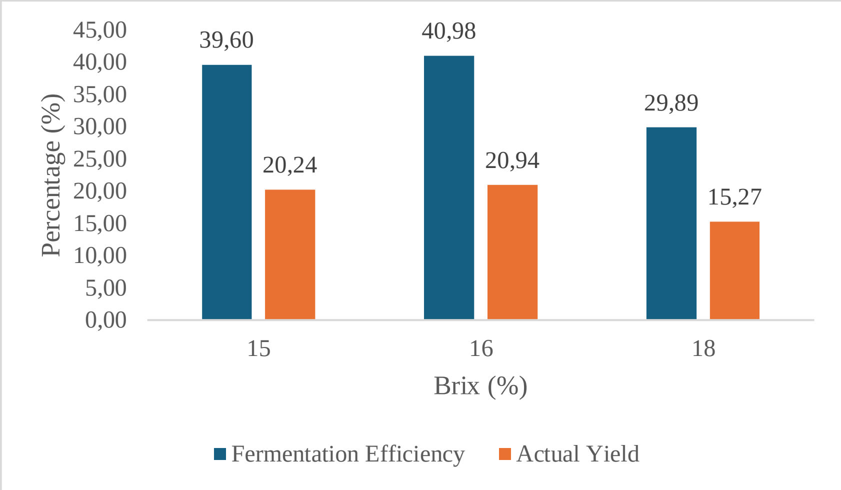

Fig. 4: Actual bioethanol yield and fermentation efficiency across the three molasses concentrations.

Despite its higher sugar conversion, 18 °Brix produced the lowest actual bioethanol yield (15.27%, efficiency 29.89%), while 16 °Brix gave the highest (20.94%, efficiency 40.98%), followed by 15 °Brix (20.24%, efficiency 39.60%) (Fig. 4). This apparent contradiction the highest sugar conversion but the lowest ethanol yield is consistent with an osmotic-stress response: under the high osmotic pressure created by a more concentrated substrate, S. cerevisiae activates the High-Osmolarity-Glycerol (HOG) pathway, diverting part of the glycolytic carbon flux toward glycerol synthesis as an osmoprotectant instead of ethanol [30]. Each mole of glucose redirected this way yields glycerol but no ethanol, so ethanol yield per unit of sugar consumed falls as the osmotic-stress response intensifies, explaining why 18 °Brix consumed the most sugar yet converted the smallest share of it into ethanol; part of the shortfall below the theoretical 0.511 g/g limit is likewise expected at every concentration, since some carbon is always allocated to yeast biomass and minor organic-acid byproducts rather than ethanol [31]. This pattern is consistent with reports that excessive substrate concentration raises the osmotic pressure of molasses media beyond the yeast's tolerance and suppresses fermentation performance [1]. On this basis, 16 °Brix represents the optimum molasses concentration under the tested conditions, while 18 °Brix shows early signs of osmotic inhibition.

4. Conclusion

The IoT-based bioethanol fermentor integrating temperature, pH, CO_2, ethanol, pressure, and bubble-count sensors on an ESP32 platform, equipped with a real-time monitoring dashboard and data-logging system, was successfully designed and implemented. The CO_2 and estimated ethanol profiles showed a qualitative rise–plateau–decline pattern across all molasses concentrations, with the fastest response observed at 18 °Brix, followed by 15 °Brix and 16 °Brix. Molasses concentration affected fermentation performance, with the highest actual yield and efficiency obtained at 16 °Brix, at 20.94% and 40.98%, respectively, followed by 15 °Brix at 20.24% and 39.60%, while the lowest values were obtained at 18 °Brix, at 15.27% and 29.89%, respectively. The optimum fermentation concentration was determined to be 16 °Brix. The temperature sensor, dashboard, and data-logging system were suitable for real-time monitoring, while the MQ-135 and MQ-3 sensors were able to indicate fermentation trends qualitatively. The pressure, pH, and bubble-counter sensors could not yet be used as reliable monitoring parameters under the experimental conditions. Quantitative analysis was performed using laboratory methods as the reference for fermentation results.

5. Abbreviations

IoT	Internet of Things
CO_2	Carbon dioxide
ZA	Ammonium sulfate nitrogen fertilizer (Zwavelzure Ammoniac)

HOG	High-Osmolarity Glycerol (osmotic-stress pathway)
GYEB	Glucose Yeast Extract Broth
MOS	Metal-oxide semiconductor (gas-sensor type)
ESP32	Microcontroller module used for sensor data acquisition
w/w, v/v	Weight per weight, volume per volume
rpm	Revolutions per minute
ppm	Parts per million

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