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Applications of Immobilized Bacillus subtilis in Bio-butanol Production: Purification and Fuel Characteristic Analysis

Neethu Asokan*
Department of Lifesciences, Sri Sathya Sai University for Human Excellence, Gulbarga, Karnataka, India.

ABSTRACT

The need for eco-friendly energy sources has led to a surge in biofuel research, where bio-butanol is emerging as a potential alternative to fossil fuels. The study delves into bio-butanol purification and fuel parameter analysis using immobilized Bacillus cells. A maximum amount of bio-butanol was recovered by integrating liquid-liquid extraction with distillation techniques. Bio-butanol production was boosted to more than 80 % compared to free cells. The immobilized cells of the potent Bacillus subtilis strain (T1) demonstrated remarkable efficiency, elevating bio-butanol production from 8.16 g/L (with free cells) to a substantial 15.03 g/L (immobilized cells). The recovered bio-butanol was suitable as a direct fuel source and a blend with standard petrol. The immobilization of the cells further solved one of the limitations in bio-butanol production. This breakthrough highlights bio-butanol potential as a sustainable and eco-friendly fuel, contributing to a more sustainable energy future. The findings provide a promising pathway toward reducing our dependence on conventional fossil fuels and mitigating environmental impact, making strides toward a greener and more energy-efficient future.

Correspondence concerning this article should be addressed to Neethu Asokan, E-mail: neethusasokan@gmail.com

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1. INTRODUCTION

In the quest for sustainable alternatives to fossil fuels, bio-butanol has emerged as a promising biofuel due to its high energy density, low greenhouse gas emissions, and compatibility with existing engines and infrastructure (Aakko-Saksa et al., 2023). Acetone butanol ethanol (ABE) fermentation emerged as a major industrial process during the 1960s; however, it was not necessarily encouraged (Jones et al., 2023). Bio-butanol production from renewable resources holds significant potential for reducing environmental impact while meeting the growing global energy demand (Algayyim et al., 2022). Among these resources, sago spent waste, a by-product of the sago palm industry, presents an intriguing opportunity for bio-butanol production. The product of ABE fermentation, especially butanol, is toxic to cells, which is a limiting factor and a challenge in ABE production. To overcome this challenge, several studies were done, such as the distillation of low titer products and combining various separation techniques with butan0l fermentation. Common integrated methods include liquid-liquid extraction, gas stripping, adsorption, and pervaporation (Khedkar et al., 2020; Liu et al., 2021). One such integration strategy is achieved in this study for enhanced butanol recovery.

Sago spent waste is abundant and underutilized, making it an attractive feedstock for biofuel production (Johnson & Brown, 2019). However, efficiently converting this waste material into bio-butanol requires a multifaceted approach that addresses the final product's fermentation process and quality. This sago waste released from industries differs from sago palm waste even though both have high starch left in the wastes. Both wastes must undergo pretreatment to release the sugars in them. In this study, cassava

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sago waste is utilized. The sago waste has a pH of about 5.2–5.6; EC of 085 ms/cm^{-1}, moisture content of 0.1–0.25 %, Total solids of 1.5–2.8 g/L; Ash content of 1.5–3.0 g/L, nitrogen about 0.5–0.6%; OC of 1.5–1.7% and OM of about 2.0–2.7%. Salwa et al. (2024) have mentioned the bio-ethanol production from pretreated sago waste. Pretreatment processes like drying, delignification, and other methods like acidification are used (Salwa et al., 2024). Sago waste left from sago industries roughly consists of about 66% starch remaining and 34% fibers and can be used for biofuel production (Rasyid et al., 2020). Chairul et al. (2023) have used saccharification and fermentation to pretreatment sago waste to produce bioethanol.

Optimization of bio-butanol production processes is essential to enhance yields and economic viability. The use of Response Surface Methodology (RSM) has gained prominence as an effective statistical tool for optimizing fermentation parameters (Wang & Zhang, 2018). RSM allows systematic exploration of various factors to maximize bio-butanol production efficiency. Several studies on optimizing medium composition for bio-butanol production using Palm Kernel hydrolysate have been conducted using ANOVA and RSM (Amin et al., 2024). Kumar et al. (2024) have used RSM with a three-factor box Behnken design to study enhanced bio-butanol production in clostridial col- cultures. Characterization of the produced bio-butanol is equally vital for assessing its suitability as a transportation fuel. Comprehensive characterization involves analyzing the physical and chemical properties, such as viscosity, octane rating, and moisture content, which are critical for engine compatibility and performance (Li et al., 2023).

This strategic combination enhanced the yield and ensured the bio-butanol product's purity and quality. The immobilized cells demonstrated their efficacy by consistently outperforming their free counterparts, underlining the effectiveness of immobilization techniques in bioprocess optimization. One of the key contributions of this research lies in the comprehensive analysis of the recovered bio-butanol’s fuel parameters. The meticulous evaluation confirmed that the bio-butanol obtained through this process meets the essential criteria for high-quality fuel. It displayed favorable characteristics, making it suitable for direct use as a standalone fuel and, importantly, as a blend with standard petrol. This dual usability is a significant advantage, offering flexibility in its application and enabling a smooth transition into existing fuel infrastructures.This study seeks to bridge these critical aspects of bio-butanol production by combining the optimization capabilities of RSM with a thorough characterization of the resulting bio-butanol. The application of immobilized cells in bio-butanol titer is evaluated along with the integration of liquid-liquid separation and distillation for enhanced separation of bio-butanol. The investigation also studies the fuel characteristics of the produced bio-butanol for its efficacy as a transport fuel.

2. METHOD

2.1 Collection and Characterization of Sago Waste

The sago pith waste was collected from local sago processing units located in Salem (11.6643° N, 78.1460° E), Tamil Nadu, India. The sago pith collected was dried, powdered, meshed and stored at room temperature. The physico-chemical characterization of waste like sugar content, pH, electrical conductivity, moisture content, total solids, total volatile solids, ash content, available nitrogen, oxygen content, osmolarity, odor, color were measured (A et al., 2007).

Pretreatment and Optimization of Waste for Sugar Recovery

Acid pretreatment

Different concentrations of acids (0.1–1.0 M) were taken for pretreatment, namely H2SO4, HCl, H3PO4, and HNO_3. The samples were then heated at varying temperatures (50–140 °C) at different times (10–40 min). Hydrolysate was centrifuged at 10,000 rpm for 10 min to separate the supernatant from the fluid, and neutralize the pH. The samples were then subjected estimation of sugars (Srinorakutara et al., 2006).

Organism and Culture Conditions

Bacillus subtilis strain (T1) were procured from Department of Microbiology, Periyar University SalemCultures of *Bacillus subtilis* strain (T1) were grown in T6 fermentation medium (KH_2PO_4- 0.5 g/L; $MgSO_4.7H_2O$- 0.3 g/L; $FeSO_4$. $7H_2O$- 0.01 g/L; $CaCl_2.6H_2O$- 6 g/L; Yeast extract- 3.0 g/L; sodium thiosulfate, $-0.5\ g{\cdot}L^{-1}$) for seed culture and incubated for 48 hr at 37 °C. The chemicals used in this study were of analytic grade and purchased from SRL, Sigma-Aldrich, and Himedia.

Bio-butanol Fermentation and Conditions

The isolate was inoculated into optimized fermentation media ($CaCO_3$- 4.5 g/L; ammonium nitrate- 3.0 g/L; sodium thiosulfate, $-0.5\ g{\cdot}L^{-1}$ with 200 g/L sago spent waste, pH 6.5) and incubated for 48 hr at 35 °C. The supernatant was collected from the fermentation setup and analyzed for butanol presence using high-performance liquid chromatography (HPLC) (Kumar et al., 2014).

Immobilization of Cells

The cells were immobilized in calcium alginate to study on the effect of butanol production and yield. Sodium alginate (2, 3 and 4%) and calcium chloride (2, 2.5 and 3%) at different concentration were taken to develop uniform firm beads. The sodium alginate solution was sterilized by autoclaving. The beads were checked for their firmness in PBS buffer for 96 hr. The cell viability of cells entrapped in the beads were also analyzed for every 24 hr up to 96 hr. The immobilized cells were incorporated in to the fermentation medium and fermented at optimized conditions (optimized sago waste concentration, optimized temperature, optimized pH, optimized electron carrier and optimized fermentation time). The butanol production and yield using immobilized cells was compared to fermentation using free cells (Santos et al., 2018).

Recovery of Butanol Using Liquid–Liquid Extraction- Distillation Method

2-Ethyl hexanol was selected for the separation of bio-butanol from the broth. The solvent was chosen for its selectivity for separating butanol. The supernatant collected after centrifugation of the fermentation broth was extracted using vigorous shaking for 45 min at a laboratory scale using a separating funnel holding an equal volume of extractant and supernatant. The extractant with butanol was collected for the distillation process to obtain pure butanol. Traditional distillation method was employed in the separation process integrated to liquid–liquid (L-L) extraction process. The extractant was distilled using a rotary evaporator, and the distilled butanol was collected through the outlet. The collected pure butanol is stored in airtight bottles for further characterization and study (Groot et al., 1990).

Characterization of Purified Product

The purified product is subjected to a characterization test to confirm its purity and identity. Gas chromatography–flame ionization detector (GC-FID) and HPLC characterized the product produced. GC-FID (Agilent, Santa Clara, CA, United States 6,890 N) analysis was performed with HP-5 5% phenylmethyl siloxane (30 mL × 0.25 mm ID × 320-µm film thickness), the inlet temperature of 180 °C with split less flow mode. The supernatant was collected from the fermentation setup and analyzed for butanol presence using HPLC.

Analysis of Fuel Parameters

Preliminary fuel test

The bio-butanol was also tested for purity using a filter paper test. The filter paper test is a simple test to check sample purity. A drop of fuel on the filter paper was observed for its evaporation. Pure fuel vaporizes, leaving the filter paper without any trace or patch of fuel (Chutke et al., 2016).

Analysis of fuel parameters

The separated purified product was checked for its purity and efficacy as transport fuel using parameters like density, calorific value and filter paper test. The combustion efficacy of the product obtained was compared to the standard petrol fuel to calculate the economic efficiency of the product at different concentrations (10, 25, 50, 75, and 100%) of the product with commercial gasoline (Blend) (Saraswat & Chauhan, 2018).

3. RESULTS AND DISCUSSION

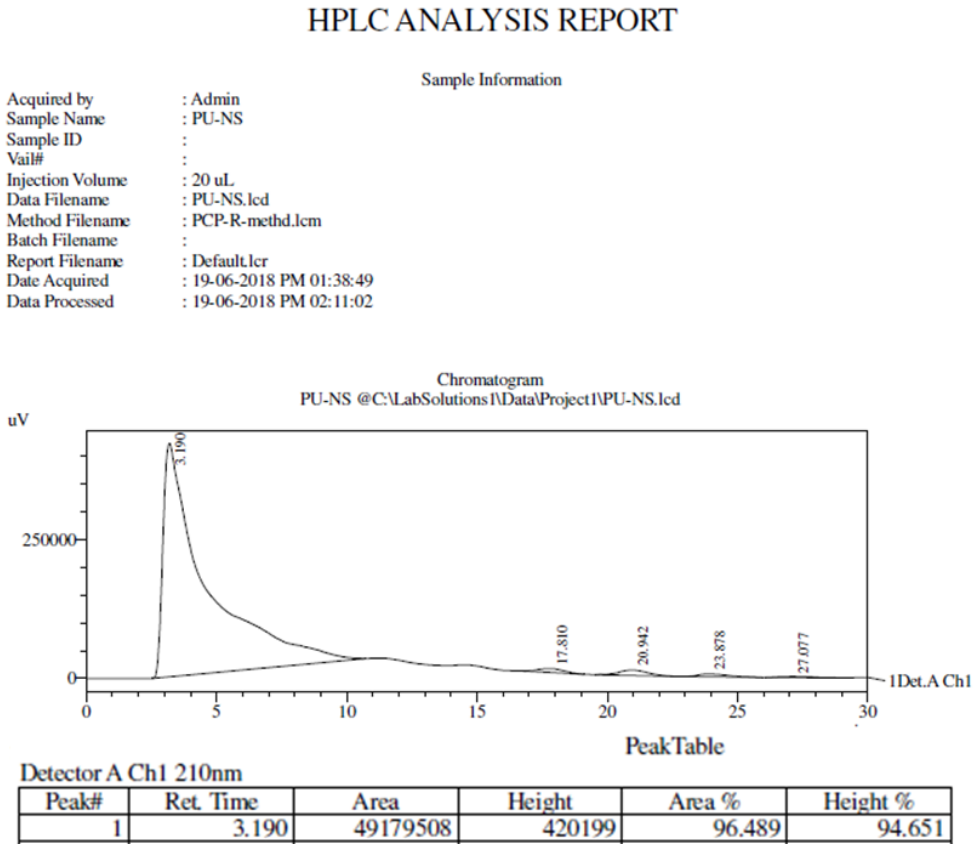


Figure 1. **High-performance liquid chromatography (HPLC) chromatogram of the fermentation sample**

Evaluation of Solvent Production

The strain was used for fermentation using standard fermentation conditions (optimized fermentation medium, 37°C, 48h) and evaluated for butanol production using sago supplemented fermentation medium (Sago supplemented (200g/L) T6 medium, 37°C, 48h). The culture produced about 8.16g/L of butanol with a productivity of 0.2g/L/h. The retention time was compared with standard butanol peak and was found to be similar (**Figure 1**). The sugar concentration in the fermentation medium was also analyzed, and it was found that sugar concentration decreased from an initial 60g/L to 5 g/L of sugar. It was found that the isolate was able to produce about 8.16g/L of butanol in sago hydrolysate medium (200g/L—eqv. to glucose: 60g/L) with productivity of 0.2g/L/h compared to butanol yield in the medium at 48h (7.2g/L butanol and productivity of 0.15g/L/h). However, the concentration was lower than the standard isolate *Clostridium acetobutylicum* (12g/L). Similar reports were also reported with the production of 10.38 g/L butanol in a batch process, and optimization of the fermentation conditions gave about 12.3 g/L of butanol (Ng *et al.*, 2014). Some other reports have stated the production of 10.5 g/L ABE at 1:4:11 Acetone: Butanol: Ethanol from cassava waste residue using facultative anaerobes (Johnravindar *et al.*, 2019). Production of butanol from sago hampas was reported to be about 4.62g/L (Husin *et al.*, 2018).

The growth curve of the *Bacillus* was studied to understand its growth under varying concentrations of sugar in the fermentation medium. It can be observed that the isolate tends to utilize some of the sugar for its growth at 24 h fermentation, and at 48 h, the isolate used up to about 55g/L of sugar in the medium reaching the stationary phase of growth. The growth curve of the isolate entered the death phase; the sugar concentration was observed to be around 3.0g/L–2.0g/L (**Figure 2**). There are previous reports of using HPLC for the detection of solvents like ethanol, acetone, and butanol similar to the current study (Buday *et al.*, 1990; Tsuey *et al.*, 2006).

Determination of Bacterial Growth

Cell biomass was estimated after 24 h for treated sago solid waste, and 0.74 OD was observed at 600 nm with UV–Vis spectroscopy. Maximum growth was observed in sago pith waste after 48–72h (1.85 OD at 600nm). The bacterial growth in a simple Thioglycollate medium was 0.60 OD after 24h. There are previous reports by Ni *et al.* (2011) discussing the effect of parameters like pH, temperature, nitrogen ratio, and chemical reactions on the negative culture growth (Ni *et al.*, 2011). They also indicated that NaOH and HCl pH adjustments could deplete cell growth. However, this study observed no growth hindrance due to alkali or acid. Ruban *et al.* (2013) have reported the efficient growth of *Bacillus sp* and *Aspergillus sp* and high amylase production in sago effluent due to its high amount of organic material. Shalinimol (2016) has reported similar competent growth in sago industrial waste.

Bio-butanol Fermentation by Immobilized Cells

Preparation of immobilized cells

Immobilization of cells using sodium alginate (2, 3, and 4%) and calcium chloride (2, 2.5, and 3%) at different concentrations revealed the use of 4% sodium alginate and 2.5% $CaCl_2$ as a better concentration for uniform and stable cell beads.

Stability and Viability of beads

The cell viability was found to be stable and active up to a month (**Table 1**). The beads (2 g) were dissolved in citrate buffer, serially diluted, and when plated onto thioglycollate plates, it was found to have approximately 3×10^6 c.f.u./ml. The cell beads were also tested for reusability. It was found that the cells could be used about thrice for fermentation with any disruption of beads. The butanol production was analyzed using immobilized cells (**Table 2**).

Table 1: Cell viability of entrapped *B. subtilis* cells

Days	1	7	14	21	25
CFU/ml	3.2×10^6	3.2×10^6	3.2×10^6	3×10^6	3×10^6

Table 2: Butanol production using different concentrations of $CaCl_2$ and sodium alginate for immobilization in fermentation medium (with ANOVA error values)

Butanol concentration g/L		
Sodium alginate	3%	4%
2% $CaCl_2$	11.9 ± 0.08	12.3 ± 0.2
2.5% $CaCl_2$	12 ± 0.1	15.03 ± 0.04
Free cells	8.16 ± 0.1 g/L	

Among the different concentrations used, it was observed that the combination of 2.5% $CaCl_2$ with 4% sodium alginate was stable and enhanced butanol production compared to traditional production of 8.16 g/L as analyzed before. Tsai et al. (2020) have immobilized *Clostridium acetobutylicum* to yield

13.80 g/L of bio-butanol from rice straw. They have used polyvinyl alcohol for the immobilization of cells to improve cell loading and protect cells from butanol cytotoxicity. Jiménez-Bonilla et al. (2022) have stated using chitosan powder to immobilize cells for enhanced bio-butanol production to 97% and improved the butanol titer up to 21%. They also compared other analog derivatives like microcrystalline cellulose, 2-chloro-N, N-diethyl aminoethyl chloride, and 3-chloro-2-hydroxypropyltrymethylammonium as immobilization carriers. There are also reports on using calcium alginate immobilized cells of *Clostridium acetobutylicum* for solvent production, which were found to increase the yield to a better extent (Häggström et al., 1980). Liu et al. (2023) have also reported that immobilization of cells can enhance bio-butanol production.

Fermentation of Bio-butanol

The potent isolate was immobilized for fermentation using the calcium alginate technique at a concentration ratio of 4% sodium alginate and 2.5% $CaCl_2$. It was observed that the concentration of butanol was higher in the fermentation medium with immobilized cells. The bacterial entrapped cells were able to produce a butanol concentration of 15.03 ± 0.04 g/L at fermentation under optimized conditions (**Table 3**). The cells could be reused two to three times. The results were better than butanol obtained from optimization of medium (12.96 ± 0.1 g/L) and traditional bio-butanol production method using free cells (8.16 ± 0.06 g/L). Similar results were reported previously, producing about 11.1 g/L of butanol from xylose with immobilized cells compared to 8.48 g/L of butanol, 28.3% higher than suspended cells (Chen et al., 2013). Krasňan et al. (2018) have also reported that entrapped cells could increase the butanol concentration by 6.3 times than free cells using pure glycerol due to the protection of microorganisms by gel matrices from the toxicity of the product. Aragão Börner et al. (2014) have reported that, unlike the free cells, the immobilized cells gave a 2.7-fold increase in butanol concentration at 18.2 g/L. They also reported that the immobilized cells were used 3 to 5 times. Häggström and Molin (1980) have reported the implementation of immobilized spores of *Clostridium acetobutylicum* in a calcium alginate gel. to obtain 67 g butanol/L-day and achieve continuous butanol production for 1,000 hr. Frick and Schügerl (1986) have also stated similar results of enhancing butanol concentration using calcium alginate immobilized cells where the ABE production increased from 1.93 to 4.02-g/L/hr productivity. The recycling of immobilized spores was reported to afford an average ABE concentration of 19.31 g/L from the third run (Shafei et al., 2002). Schoutens et al. (1985) have performed similar studies of calcium alginate cell immobilization of *Clostridium* spores to produce butanol continuously from glucose with reactor productivities of 1.0–4.0 $kg/m^3/hr$ and 0.22 $kg/m^3/hr$ with immobilized cells and free cells respectively.

Table 3 Bio Butanol Production at Different Fermentation Conditions Using ANOVA

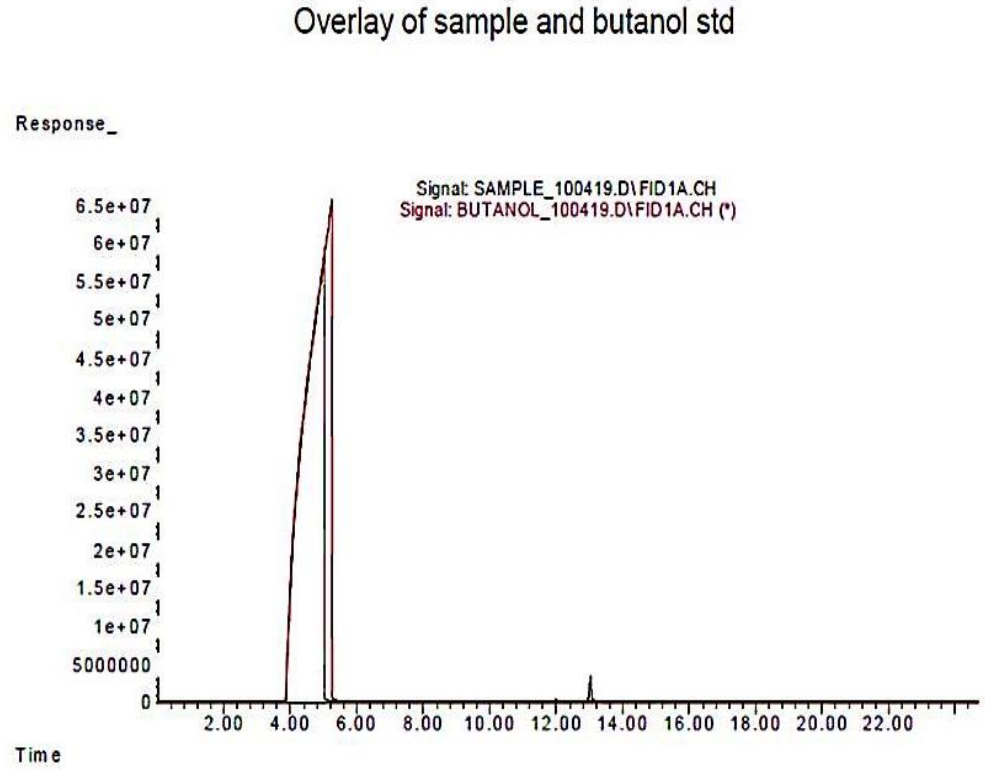
	Optimized medium		Standard medium	
	Immobilized cell	Free cell	Immobilized cell	Free cell
Butanol concentration	15.03 ± 0.04	12.96 ± 0.1	10.06 ± 1.4	8.16 ± 0.06
Reduced sugar	0.0258 ± 1.2	0.0380 ± 0.85	0.0330 ± 0.15	0.0434 ± 1.2

Recovery of bio-butanol

Integration of liquid-liquid extraction and Distillation for butanol recovery

It was found that approximately 12 g/L of butanol could be extracted from 24 hr optimized fermentation setup and approximately 15 g/L from 24 hr immobilized fermentation setup. Ester and Pelarut (2015) have previously reported 2 ethyl 1 hexanol as the best extractant due to increased contact surface area. Liu et al. (2004) and Liu et al. (2021) have also used 2-ethyl 1 hexanol to separate solvents from fermentation broth to the extractant phase. Chen et al. (2021) have reported using solvents to separate bio-butanol from the fermentation medium. Their study implemented liquid-liquid separation with oleyl

alcohol for butanol separation. González-Peñas et al. (2020) demonstrated the strategy of liquid–liquid separation using two butyl-1-octanol for butanol separation. Dalle and Adams. (2018) compared various toxic and nontoxic extractants like decanol, hexanol, mesitylene, 1:50 wt% decanol:oleyl alcohol, 2:20 wt% decanol:oleyl alcohol, 2-ethyl 1 hexanol and oleyl alcohol, 2-ethyl 1 hexanolwas reported to be promising extractant with 99.8% butanol recovery due to its high butanol distribution coefficient.



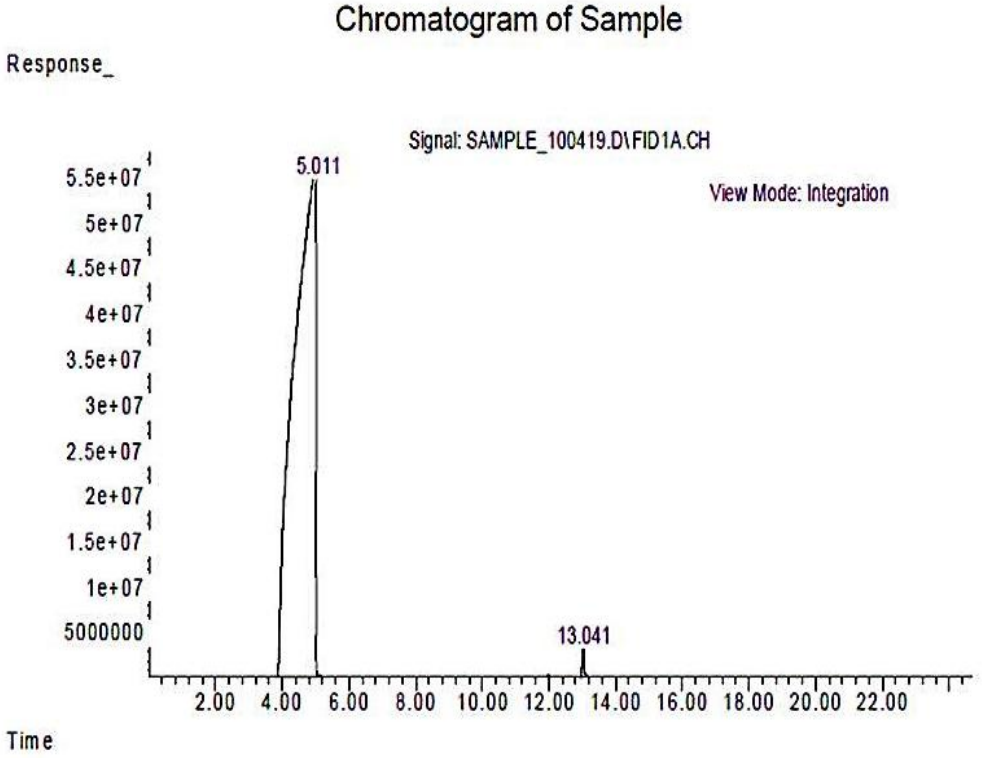


Figure 2. Gas chromatography–flame ionization detector (GCFID) chromatogram showing peak of purified butanol (top) and overlay of standard and sample (bottom)

Dadgar and Foutch (1988) have also stated the corresponding results to the present study stating 2-ethyl 1 hexanol as a better extractant to remove ABE from water. Van der Merwe et al. (2013) has also reported similar reports of using 2-ethyl 1 hexanol for extraction of bio-butanol. The extraction was followed by distillation. Distillation of the extractant with butanol separated purified butanol and recovered 90% of extractant- 2-Ethyl hexanol, which was reused for extraction. The extractant thus obtained could be reused twice. The separation process was more economical than the traditional distillation of fermentation broth. About 15.5 g/L butanol was distilled from immobilized cells mass fermentation at 24 hr, and about 120–150 ml of extractant (2-Ethyl hexanol) was recovered from 200 ml used. The continuous fermentation under the same conditions and reusing the immobilized cells and extractant, a total of 31 g/L of butanol was attainable at 48–96 hr of fermentation. Research has shown that liquid-liquid extraction integrated with distillation is one of the best-adopted methods due to its simplicity and better recovery of butanol compared to other techniques like distillation (<20 g/L in the reactor) and pervaporation (Qureshi & Blaschek, 1999) giving >75 g/L in the reactor when combined with gas stripping, which is costly with *Clostridium sp* (Xue et al., 2015). Liquid-liquid extraction have reported to giving ABE concentrations of 23.8 and 25.3 g/L with whey permeate and glucose, respectively, using *Clostridium sp* (Qureshi et al., 1992; Roffler et al., 1987).

Characterization of the Purified Butanol

GC-FID

The sample analyzed with GC-FID (Agilent 6,890 N) gave sharp peak at retention time 5.011 and a minute peak at retention time 13.041. The sample was compared with standard butanol (Sigma, HPLC grade) and the retention time observed was similar to sample (RT 5.253) and minute peak was identified as 2-Ethyl hexanol (**Figure 2**). Potter et al. (1996) have also reported similar results of GC-FID analysis for petroleum and butanol. The present results are supported by the results reported by Lin et al. (2014) to validate the ABE fermentation products. Owens and Lowe (2023) have reported on the analysis of fusel oils that include n-butanol using GC-FID with a validated method using a headspace sampling unit.

Analysis of Fuel Parameters

The purity of the fuel was confirmed by a basic filter paper test. A drop of butanol purified, and petrol was added to the filter paper and observed for evaporation. The butanol purified vaporized in 60 seconds while petrol vaporized at 40 seconds, leaving the filter paper without any trace of fuel. This confirmed the purity of the sample as similar to petrol (**Figure 3**). There are previous reports of butanol-fuel analysis for the fuel application in internal combustion engines (Hönig et al., 2014). Jenkins et al. (2013) have also reported the importance of fuel parameters for selecting biofuels for road and aviation sectors. Previous studies on fuel blends have reported corresponding analysis results of 5 and 10% butanol blends (Rakopoulos et al., 2011). Feng et al. (2013) have proved the possibility of 35% butanol blend with gasoline.

Table 4 Fuel Parameter Analysis of Bio-butanol

Sl no	Properties	Butanol
1.	chemical formula	C_4H_9OH
2.	density in kg·dm^{-3} at 15 °C	0.81
3.	viscosity in mm^2/s	3.61
4.	boiling point °C	117
5.	calorific value MJ/kg	38.5
6.	heat of vaporization MJ/kg	0.44

7.	vapor pressure kPa	18.2
8.	research octane number	90
9.	oxygen content %	20.6

The fuel parameters of bio-butanol, namely Chemical formula, Density in kg·dm^{-3} at 15 °C, Viscosity in mm^2/s, boiling point degrees Celsius, Calorific value in MJ/kg, Heat of vaporization in MJ/kg, Vapour Pressure in kilopascals, Research Octane Number and oxygen content in % were studied (**Table 4**) and compared with Gasoline parameters.

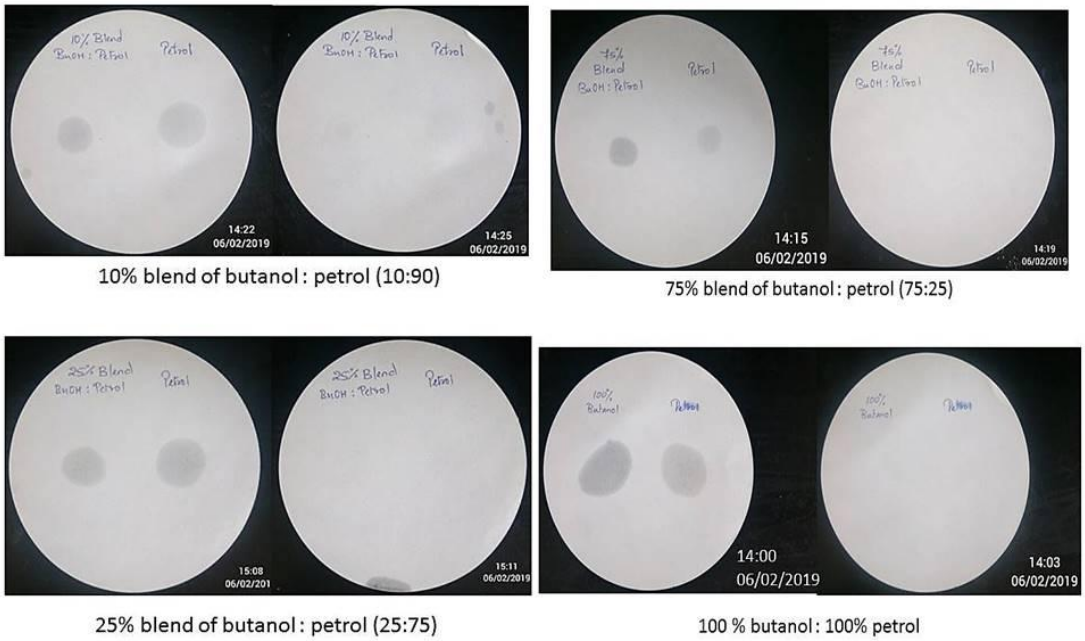


Figure 3. **Filter Paper test for purity analysis of fuel blend**

Efficiency of purified product as a commercial fuel blend

The efficiency of blended fuel was tested using two significant basic parameters, namely the gross calorific value and density. The purified sample was blended with commercial petrol and analyzed for calorific value and density to understand better-blending proportions. The more similarity of the Calorific value and Density to the Gasoline value, the better the sample application as a transport fuel. The reports obtained in the present study were similar to previous reports (Hönig *et al.*, 2014). The purified sample alone was also analyzed to identify the efficiency of pure bio-butanol as fuel. The results obtained are schemed in **Table 5**.

Table 5: **Fuel Blend Analysis Report Analyzed from Polytest Laboratories, Mumbai**

Sl no	Fuel blend	Calorific value (MJ/kg)	Density at 15 °C (kg/dm^3)
1	**a (10% bio-butanol)**	35.9	0.764
2	**B (25% bio-butanol)**	36.6	0.766
3	**C (50% bio-butanol)**	29.5	0.791
4	**D (75% bio-butanol)**	33.5	0.809
5	**E (100% bio-butanol)**	38.52	0.8173
6	**petrol (Indian oil)**	40.0–45.0	0.71–0.77

It was observed that the better blend with respect to calorific value analysis was 10–25% bio-butanol blend followed by the direct usage of 100% bio-butanol. Similar reports were previously reported corresponding to present study (Chen et al., 2013). However, the density of the blend can be observed in ascending manner indicating the purity/quality of fuel, effect of specific gravity and blending proportion. Linggang et al. (2013) have similarly reported the production of ABE using sago pith as transport fuel. Crabbe et al. (2001) have also given supportive reports stating the butanol fuel from sago pith waste and its fuel properties. It can be derived that the direct use of pure bio-butanol could be a better choice for transport fuel.

4. ECONOMIC ANALYSIS

The biofuel derived from sago waste is projected to cost approximately Rs 54/L (approximately $0.8/L), making it highly cost-effective when compared to conventional petrol priced at Rs 73.36/L ($1.02/L) as of December 2018 (using Microsoft® Excel 2010). Previous studies evaluating bio-refinery methods have shown varying yields of bio-ethanol per kilogram of biomass: 0.16, 0.17, 0.22, 0.19, and 0.14, respectively (Demichelis et al., 2020). A recent study by Joseph et al. (2023) revealed a total capital investment of approximately US$17/gal. for bio-ethanol production from sugarcane bagasse and US$12 for brown algae processes.

5. CONCLUSIONS

In conclusion, this study illuminates a promising avenue for sustainable energy production through enhanced bio-butanol production, purification, and comprehensive fuel compatibility analysis. By integrating immobilized Bacillus subtilis cells with advanced purification techniques like liquid–liquid extraction and distillation, we overcame some common limitations in ABE fermentation. The immobilized cells were able to produce the bio-butanol to 15.03 g/L, a notable improvement compared to the 8.16-g/L achieved with free cells. As the global community faces the pressing challenges of climate change and depleting fossil fuel reserves, bio-butanol emerges as a beacon of hope. The ability to harness the metabolic capabilities of specific strains, as demonstrated by Bacillus subtilis (T1), showcases the potential of microbial biotechnology in addressing our energy needs. In conclusion, this research contributes valuable knowledge to the scientific community and offers practical solutions for transitioning toward a greener energy landscape. One significant factor is the ability of facultative anaerobes to produce bio-butanol under conditions. This ability can be further exploited in a positive route for biofuel production, thus cutting the cost of culture maintenance. We have taken a significant step toward a more sustainable future by harnessing the power of bio-butanol and innovative bioprocessing techniques.

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