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Application of Submerged Fermentation for Production of Biobutanol from *Colocasia esculenta* (L.) Schott (Talas Liar) Peels

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Abstract

The recent work has focused on the production of biobutanol from *Colocasia esculenta* peels waste using a pure strain of *Clostridium acetobutylicum* ATCC 824 at different concentrations of 20, 40, and 60 g/L of peels as a rich source for carbon for the fermentation process. The maximum carbohydrate content (48.16 g/L) was attained at 60 g/L of the peels. The higher biomass growth of the investigated strain (10.5 g/L) was achieved when 60 g/L of the peels were used at 72h. By increasing the peels concentrations from 20 to 60 g/L, where the resulted biobutanol had elevated from 2.11 to 12.56 g/L respectively at 72h. The process of fermentation parameters was optimized where the best pH and fermentation temperature values were 6.5 and 35^{o}C respectively in the presence of 60 g/L of peels at 72h.

Keywords: Bioutanol; *Colocasia esculenta* peel; Gas Chromatography Mass Spectrometry; *Clostridium acetobutylicum* ATCC 824.

1. Introduction

For the few past decades, fuel from fossil was used as the main energy sources over many countries **[1]**. The uncontrolled consumption of the fossil fuel, for several years, with increasing the human awareness towards the environmental pollution has led to searching for sustainable and natural alternatives to the traditional fossil fuel **[2]**. Biofuel production especially fuel alcohol from agricultural wastes has of a great interest over the last years [**3**]. The most commonly consumable biofuels were found to be acetone butanol and ethanol (ABE) [**4]**. In a comparison to bioethanol, biobutanol has various advantages where it is high in energy content with less corrosive effect **[3]** (which give the hope for potential butanol distribution through pipelines without the need for usual transportation (through trucks or railways which is already used in bioethanol) could be used individually or together with gasoline and could be worked efficiently in engines without the need for any modification **[5, 6]**. Butanol also has a non-hygroscopic nature which subsequently give it a longer shelf life [**7**]. On the other hand, biobutanol resemble bioethanol in which both help to reduce CO levels in vehicles exhaust where it is a good source of oxygen [**3**]. Butanol (4-carbon alcohol) is a major biofuel material used in the manufacturing of a variety of chemical products which sometimes have properties superior to those of ethanol [**7**]. Biobutanol has many applications as a solvent in various clinical as well as industrial fields such as antimicrobials, vitamins, hormones, chemical intermediates, paint thinner processing, and hydraulic and braking fluids manufacture [**6, 8, 9, 10, 11].** Biobutanol dissolves completely in a variety of organic solvents [**5].** Biobutanol production from agricultural (rich in lignocellulosic materials) wastes has encouraged many researchers to avoid the uses of food of edible source for the production of such biofuels **[12, 13]**. Wastes of agricultural origin such as rice husk, rice, barley as well as wheat straw, sugarcane bagasse and different agricultural wastes that considered as an important resource for biofuel production **(14, 15).** Generally, agricultural wastes are organic substances that are produced from the agricultural harvests and can be used as a biofuel substrate alternative [**14**]. These wastes could be classified as agricultural crop residues and agro-industrial wastes [**16**]. The former

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comes from plant origin that left after harvesting of crops, while the other are the results of the post-harvesting procedures like cleaning, sieving and sorting [**1]**. Due to scarcity of crop residues, where they are used as fertilizers and as animal feed, the agro-industrial wastes were used instead as they are largely collected from the field sites and also, they provide low costs for handling and transportation processes [**17].** Cellulose, hemicellulose, and lignin are the main components of the cell wall composition in the agricultural wastes [**1]**. Particularly, biobutanol could be achieved using different anaerobic microorganisms to ferment a variety of carbohydrates such as sucrose, cellulose, glucose, fructose, mannose, lactose, dextrin, starch, xylose, and arabinose [**18**]. A variety of microorganisms had shown their abilities to produce biobutanol most of them are related to the *Clostridium* species. These species have distinct properties represented in their abilities to consume different carbon sources, which gave them the priority for biobutanol production. The most favorable candidates as solvent producers belong to the genus *Clostridium spp.* Including, *C. acetobutylicum, C. beijerinckii, C. saccharobutylicum*, and *C. saccharoperbutylacetonicum*. They are all spore-forming anaerobic bacteria [**19, 20].** *C. acetobutylicum* performs many industrial applications through a unique metabolic pathway, mainly, including the production of organic solvents, such as acetone, butanol, and ethanol [**21-23**]. Cells of *Clostridium acetobutylicum* are rods in shape with diameter of 0.5–0.9 × 1.5–6 μm. They are motile, stained as Gram-negative in older cultures and their spores are oval and subterminal [**24**].

The present study aims to use cheap agricultural wastes that would serve as sole carbon source for production of biobutanol using microbial fermentation. This method would not only enhance the over production of biofuel constraints, but also considered as promising alternatives to reduce the accumulation of agricultural wastes.

2. Materials and methods

Colocasia Peels collection

Colocasia esculenta peels (CEP) were collected during the period between September and October 2020 from local markets in Jeddah (West of Saudi Arabia). Approximately 100g of CEP were washed with distilled water (several times) and dried using an oven for 48 h at 60°C to avoid fungal growth as well as bacterial invaders. Then the dried CEP was ground in the blender to a mesh size between 0.2 and 0.4 mm using stainless steel sieves and kept in sterilized plastic containers for further studies.

Substrate preparation

Prior to fermentation process, the CEP (w/v) in 3 different concentrations of 20, 40, and 60 g/L were boiled in a water bath (100°C) for 60 minutes, allowed to be cooled, and filtered through a gauze. The three filtrates of CEP were analysed for their total carbohydrate content using anthrone method [**25**].

Chemical analysis of CEP

The total carbohydrate content in CEP (60g/L) was quantitatively determined by anthrone method [**25**]. Moisture content, fat content, protein content and ash were determined according to Association of Official Analytical Chemist (AOAC) official methods. All experiments were carried out in three replicates.

Microorganism and inoculum preparation

Clostridium acetobutylicum ATCC 824 was kindly dedicated from the Microbiology Laboratory, Biology Department, College of Science, University of Jeddah, Jeddah, Saudi Arabia. *C. acetobutylicum* was previously stored in a re-inforced clostridial broth medium (RCM, Oxoid, Basingstoke, UK) with glycerol (20%, v/v) at −80 °C. The RCM contained (g/L): peptone 10, yeast extract 3, beef extract 10, NaCl 5, soluble starch 1, dextrose 5, cysteine hydrochloride 0.5, sodium acetate 3 and agar 0.5. All the components were dissolved in distilled water up to 1L and sterilized at 121°C for 15 minutes. Prior to fermentation, 1 mL of *C. acetobutylicum* ATCC 824 stock suspension was transferred to 99 mL of RCM and N2 gas was sparged for 15 min before sterilization at 121°C for 20 min. Then the culture was incubated at 37°C for 48 h under anaerobic culture conditions to enhance the spore formation **[26]**. The growing cells under anaerobic conditions were transferred into fermentation bottles for further studies.

Biobutanol production in submerged fermentation

The filtrates of CEP were further studied for production of biobutanol by *C. acetobutylicum* ATCC 824. The filtrates of CEP (rich in sugars) were supplemented with 5 g/L of yeast extract, 2 g/L of NH_4Cl, 5 g/L of $CaCO_3$ and P2 stock solution [**27**]. In a batch fermentation the solvent production was carried out in 125 ml flasks containing 90 ml of CEP filtrates with N2 gas (99.99%) for 5 min to enhance anaerobic condition. pH of the mixture was achieved at 6.5 using 1M NaOH and 1M HCl and autoclaved at 121°C for 15 minutes. After cooling, inoculum of *C. acetobutylicum* ATCC 824 at concentration of10% (v/v) was added to the medium under aseptic conditions before the onset of fermentation process and the bottles were incubated at 37°C for 120 h. Biobutanol production from CEP filtrates was compared with 20, 40, and 60 g/L. Samples of 2 ml of each batch were taken at different periods (24 h) for further analyses. Prior analyses, the samples were centrifuged at 10,000 rpm for 10 minutes at 4°C.

Analytical methods

The cell growth was estimated by measuring optical densities (ODs) at 600nm [**28**] using UV-Visible spectrophotometer (Shimadzu UV-2450). The biobutanol production was analysed by Gas Chromatography with Mass Spectrometer (Shimadzu QP 2010 Ultra GC-MS) with flame ionization detector (FID) after centrifugation of samples for 20 minutes at 10,000 rpm and 4°C [**29**]. The solvents were separated on Stabil wax capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) using helium as the carrier gas with a flow rate of 3 mL/min. A split ratio of 10:0 was used. The detector and injector temperatures were set at 180 and 220°C. The oven temperature was increased from 40 to 190°C at the rate of 20°C/min to 220°C. The calibration curve of butanol was prepared with GC grade standards of butanol with concentrations of 20, 30, 40, and 50ng/mL. Consequently, pH values were adjusted using pH meter (Hanna instruments) parallel to biobutanol production during the fermentation process. Biobutanol yield and Biobutanol productivity were calculated using the following equations:

Biobutanol yield (g/g) = Total biobutanol produced (g/L) / Total carbohydrate utilized (g/L)

Biobutanol productivity (g/L/h) = Total biobutanol produced (g/L) / Fermentation time (h).

Optimization of fermentation parameters

Initial pH

effect of initial pH of the medium on biobutanol production by *C. acetobutylicum* ATCC 824 from CEP filtrate (60 g/L) was studied. The initial pH values varied from 4.5 to 7.5 were tested to investigate the appropriate pH value for biobutanol production.

Temperature

effect of incubation temperature on biobutanol production by *C. acetobutylicum* ATCC 824 from CEP filtrate (60 g/L) was investigated. Different temperature values varied from 25 to 40ºC were tested to investigate the appropriate incubation temperature degree for biobutanol production.

3. Results

The CEP used in the present study had a considerable sugar content in its peels. The total carbohydrates content from 20 g/L filtrate was 21.72 g/L (**Figure 1**) which resemble the water-soluble fraction in CEP waste. The total carbohydrate content further increased with increasing the peels concentrations, where, in 40 g/L and 60 g/L the sugar contents were 34.22 g/L and 48.16 g/L respectively. The sugar content was 1.58 times and 2.22 times for 40 g/L and 60 g/L of CEP respectively when compared to 20 g/L.

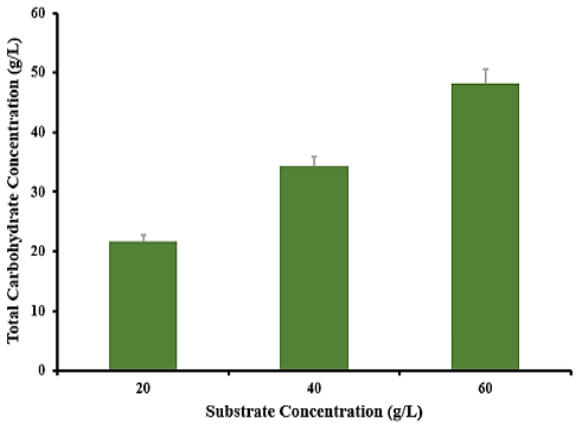

Figure 1. Total sugar content from different concentrations of CEP

The CEP with mesh size 0.2 to 0.4 mm was provided in the present experiment. Its moisture content, fat, protein, total carbohydrate content as well as its ash content were chemically determined as shown in **Table 1**. The proximate analyses showed that CEP were rich in total carbohydrate content which gave the hope to investigate these wastes as source for biobutanol production.

Table.1 Composition of CEP

Constituent	CEP*
Moisture content	7.6±0.21
Fat	0.76±0.54
Protein content	7.2±0.61
Total carbohydrate content	48.16±0.28
Ash content	2.1±0.73

*Values were expressed in terms of percentage ± standard deviation based on dry weight.

The effect of CEP filtrate on bacterial biomass concentration of *Clostridium acetobutylicum* ATCC 824 was investigated for 20, 40, and 60 g/L of CEP. Data represented graphically in **Figure 2** showed that the biomass growth of the bacterial strain was gradually increased by increasing in the CEP concentration. The maximum biomass growth was attained at 72h for all the studied concentrations that was obviously followed by a decline phase. The higher concentration of CEP at 60 g/L increased the biomass growth rate of *Clostridium acetobutylicum* ATCC 824. From the results it was noticed that the biomass production increased from 6.8 g/L to 7.5 g/L to reach its maximum production of 10.5 g/L (for 20 g/L, 40 g/L and 60 g/L of CEP respectively). The maximum biomass production was achieved at 72h (60 g/L of

CEP) with 1.54 times the concentration of CEP at 20 g/L.

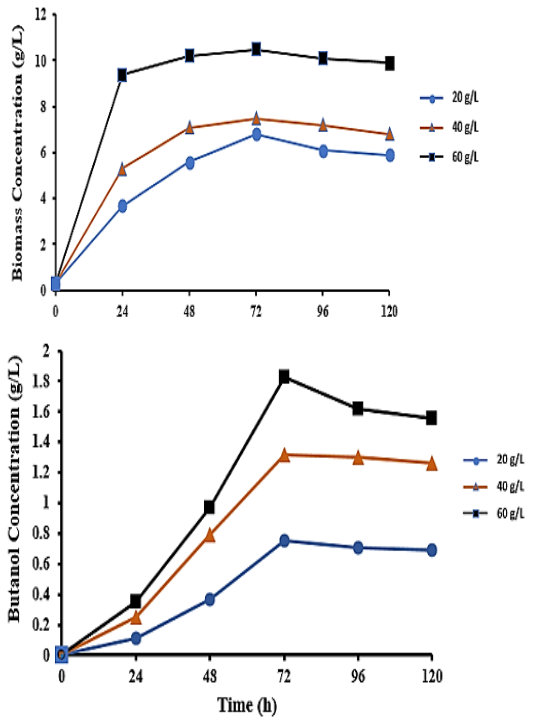

Figure 3. Maximum butanol production from different concentrations CEP (20, 40, and 60 g/L) at different fermentation incubation times.

The production of biobutanol from CEP filtrate in different concentrations (20, 40, and 60 g/L) was studied in the present work (**Figure 3**). The maximum biobutanol production was achieved at 72h. There was an increase in biobutanol production by increase of CEP concentrations. The maximum biobutanol production was 2.11, 7.22, and 12.56 g/L for CEP concentrations of 20, 40, and 60 g/L. The increase in biobutanol production was 3.42 times and 6.0 times for 40 g/L and 60 g/L respectively when compared to 20 g/L CEP.

The biobutanol yield of 0.11 g/g (with biobutanol productivity of 0.03 g/L/h) from 20 g/L CEP increased to 0.18 g/g (with biobutanol productivity of 0.10 g/L/h) for 40 g/L CEP. The yield of butanol was improved to 0.21 g/g (with biobutanol productivity of 0.18 g/L/h) for 60 g/L CEP at 72 h (**Table 2**).

Table 2. Biobutanol Yield and Productivity from different CEP concentrations (20, 40, and 60 g/L) at different fermentation incubation times.

Substrate concentration (g/L)	Biobutanol Conc. (g/L)	Biobutanol Yield (g/g)	Biobutanol Productivity (g/L/h)

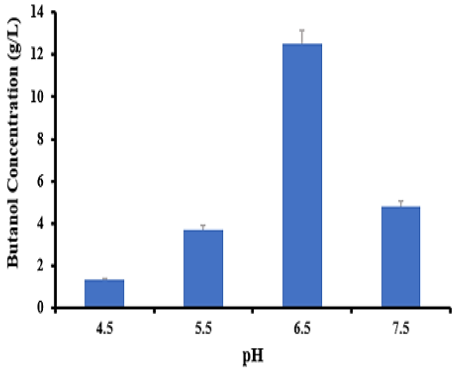

Figure 4. Effect of pH on biobutanol production from CEP (60 g/L) by *C. acetobutylicum* ATCC 824

The most suitable pH value of the medium was studied to investigate its effect on biobutanol production using fermentation process of CEP filtrate (60 g/L) by *C. acetobutylicum* ATCC 824. Different initial pH values of 4.5, 5.5, 6.5 and 7.5 were tested to investigate the appropriate pH value for biobutanol production (**Figure 4**). As shown in Table 3, the highest amount of biobutanol was achieved at pH 6.5, with 12.53 g/L. Below and above this pH degree the biobutanol production was found to be lower while the lowest biobutanol production was attained at pH 4.5 (1.34 g/L).

As shown in **Figure 5**, the effect of different fermentation temperatures on the production of biobutanol from CEP filtrate at 60 g/L was studied. It was observed that the biobutanol production increased by an increase in temperature at 35°C. The maximum production was 12.65 g/L while the lowest production was achieved at 25°C (4.73 g/L).

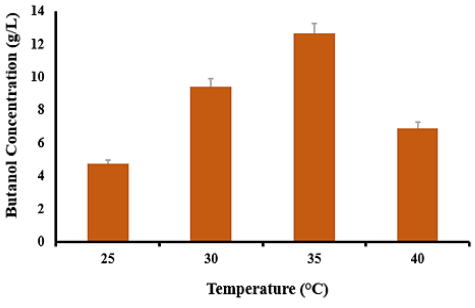

Figure 5. Effect of temperature on biobutanol production from CEP (60 g/L) by *C. acetobutylicum* ATCC 824

4. Discussion

The present study showed that *Colocasia esculenta* peels had a positive effect on biomass growth as well as biobutanol production. The increase in biomass concentration of *C. acetobutylicum* ATCC 824 increased with CEP concentrations. This result is in accordance to results obtained by **Kamboj and Ms [30]** who studied the production of biobutanol from potato peels mixed with diluted extract of orange peels by *Clostridium acetobutylicum* MTCC 11274. They also stated that the orange peel extract had a positive effect on potato peel waste in cell growth, total solvent, and organic acid production. Similarly, **Nasrah et al. [31]** investigated the production of biobutanol from *C. acetobutylicum* ATCC 824 using oil palm frond juice.

At the present work, the maximum biobutanol production (12.56g/L) was attained at 72h at 60g/L of CEP filtrate. Different CEP concentrations were studied (20 and 40 g/L) where they showed lower biobutanol production when compared to the higher CEP concentration (60 g/L). The obtained result is higher than what achieved by **Arifin et al. [32]** who stated that the production of butanol in the range of 1–2 g/L from agricultural by-products. On the other hand, the present result is close to results of **Raganati et al. [4]** who studied the production of biobutanol from feedstock peels. They found that high butanol production (approximately 14g/L) was resulted when feedstock peels were mixed with water in a 1/8 ratio. They stated that ratios less than one-sixth were characterized by low butanol concentration.

The biobutanol yield was improved from 0.11g/g where the productivity was 0.03g/L/h for 20g/L CEP filtrate to 0.21g/g and the biobutanol productivity was 0.18g/L/h for 60g/L CEP at 72 h. Accordingly, **Khamaiseh et al. [33]** mentioned that higher concentrations of biobutanol (11g/L) was attained at 50 g/L when different concentrations (10 to 50g/L) of date were used. They stated that when low concentrations of initial sugars are used, low biobutanol production was achieved and this might be because the presence of low carbon sources that could be consumed during the fermentation process. The present result is also in agreement of **Nasrah et al. [31]** who studied the total sugars in oil palm frond (OPF) juice which varied from 10 to 60 g/L.

They stated that, at 50 g/L of sugars in OPF, a yield of 0.24 g/g of biobutanol was obtained. Similarly, **Komonkiat and Cheirsilp [34]** discovered that biobutanol production from oil palm sap increased from 5.93 to 12.76g/L at 30g/L and 50g/L sugar concentration respectively.

The optimum pH for biobutanol production (12.53 g/L) was found to be 6.5 at 37ºC when 60 g/L of CEP filtrate was used in medium. This result was similar to observations obtained by **Ibrahim et al. [35]**, who discovered that the highest concentrations of acetone, butanol, and ethanol (ABE) solvents were achieved at pH 6 when compared to pH 5, 5.5, and 6.5 in the study. **Al-Shorgani et al. [2]** also stated that when the initial pH was around 6.2, the maximum biobutanol production was attained (6.28 g/L). This finding may be explained as previously mentioned by **Chua et al. [36]** who stated that the initial pH between 5.5 and 6 was the best value for solvent production. They mentioned this may be because these values of pH could enhance the biomass growth without induction of acidic environment which may inhibit the production process.

From the provided study it was noted that the maximum biobutanol productivity (12.65g/L) was attained at 35ºC while the lowest production was obtained at 25ºC (4.73g/L). **Kamboj and Ms [30]** enhanced the production of biobutanol from *C. acetobutylicum* MTCC 11274 using potato peels in diluted extract of orange peels at 37ºC.

5. Conclusions

It is concluded that *C. acetobutylicum* ATCC 824 showed a good and acceptable production of biobutanol from *Colocasia esculenta* peels which are rich carbon source wastes that also give the hope to avoid the use of corrosive chemicals and the other compounds that may affect the fermentation process and the environment as well. The present study suggests the using of solid wastes as starter compounds to reduce the cost of butanol production from when CEP was used.

6. Conflict of interest

The authors have no conflict of interest

7. Acknowledgments

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