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Cassava Roots: Perspectives of a Traditional Staple for Bio-solvents Production

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Abstract

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The culture media prepared from mashed cassava and cassava starch were used to test their convertibility into organic solvents such as acetone, butanol, and ethanol. The trials were conducted in Erlenmeyer flasks containing various culture media with initial starch concentrations ranging from 30 g/l to 100 g/l. Fermentation was carried out by *Clostridium acetobutylicum* DSM 1731. The purpose of these tests was to determine the initial starch concentration which could be used as a starting point for the fermentation in laboratory bioreactors. In this case, we used two culture media: the first one made with cassava starch (M1), and the second one with mashed fresh cassava (M2). The culture media were supplemented with organic and inorganic nutrients. Fermentation in M1 medium resulted in the production of 17.8 g/l solvents, i.e. 12.3 g/l butanol, 3.7 g/l acetone, and 1.8 g/l ethanol, while in M2 it provided 16.2 g/l solvents representing approximately 11.4 g/l of butanol, 3.0 g/l of acetone, and 1.8 g/l of ethanol. The optimum production of butanol in M1 was 12.3 g/l in 96 h of fermentation, which is a low yield of 25% and 0.13 g/l/h productivity. Although the fermentation in medium M2 was also low (with 11.4 g/l butanol optimally), it allowed to save on the nutrient supply since it was done without the addition of minerals and organic substances.

Keywords: cassava starch; *Clostridium acetobutylicum*; acetone; butanol; ethanol; organic acid; lactic acid, acetic acid; butyric acid

Abbreviations: M1 – medium composed of cassava starch + mineral nutrients and yeast extract; M2 – medium composed of mashed cassava without nutrients addition; MCSL1 – medium composed of cassava starch + mineral nutrients + corn steep liquor; MCSL2 – medium composed of mashed cassava + mineral nutrients + corn steep liquor; MYE1 – medium composed of cassava starch + mineral + corn steep + yeast extract; MYE2 – medium composed of mashed cassava + mineral + corn steep + yeast extract; CSL – corn steep liquor; YE – yeast extract; (±) approximation of the calculated concentrations

Cassava is a tropical plant occurring in the humid tropics and semi-arid areas. The geographical area of the cassava production covers the tropics of both Americas as well as Asia, and from northern Australia to the African tropics. Cassava reproduces by budding and provides at the end of the reproductive cycle it provides large tuberous roots in which significant reserves of starch are stored. The starch content in tubers of fresh cassava ranges from 25% to 30% wt (Ajao & Adegun 2009). Cassava starch is composed of about 17–24% amylose and 76–83% amylopectin. Cassava is a commodity consumed by a large number of people, mainly in Latin America, Africa, and Asia. The World annual cassava production is estimated at about 255.7 million t (in the form of tubers) (Food Outlook – FAO 2013). Africa ranks first with 53.7% of the world production, followed by Asia with 33.0%. South America ranks third with

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12.7%, while in Oceania is the production estimated at about 0.6% of the world production each year. Cassava chips, flour, starch, gari (dried small particles resulting from the processing of cassava), and tapioca (pellets of dried cassava starch) are the main cassava products. Among them, cassava starch is the most coveted because it is widely used in areas such as fermentation industries, food and pharmaceuticals, paper and textiles. The cost of cassava starch is relatively low in the world markets. The quotation of cassava starch in the world markets fell from 507 in 2010 to 489 in 2011 and 439 in 2012, before rising to 472 USD/t in 2013 (Food Outlook – FAO 2012, 2013). Economically, cassava starch is attractive, competitive if compared to potatoes and corn starches. In 2013, the quotation of cassava starch in the international market was approximately 472.2 USD/t, while corn starch was worth between 480 and 550 USD/t (Food Outlook – FAO 2013). Starch is the most commonly used raw material for ethanol production. However its cost in the fermentation process represents an important part of the production cost which, by extension, affects the cost of the end products. For example, the share of corn starch in the production costs reaches up to 79% of the total solvent production cost while the cost of energy including the downstream processes represents 14% of the total costs (Green 2011). Cassava starch applications can therefore contribute to the reduction of the cost of industrial production. Cassava starch is already used in areas such as the food conservation, production of glucose syrup, textile industry, pharmaceutical industry, paper industry, plastic industry, etc. Cassava starch hydrolysate is also much used in the production of ethanol using yeast. The conversion of ethanol from cassava is about 180 l/t of cassava or 2070 l of ethanol/ha of cultivated cassava (FAO 2008). In contrast, the production of butanol from cassava starch is not sufficiently developed as compared to the ethanol production. The fermentative butanol production began with the work of Louis Pasteur who discovered the first bacterial strain in 1861. Between 1912 and 1916, Dr. Chaim Weizmann was able to isolate the strain of the bacterium that efficiently produces solvents (Jones & Woods 1986; Dürre 2008). Initially termed as the organism Weizmann, the bacterium has become over time *Clostridium acetobutylicum* ATCC 824.

Butanol is an important chemical with many industrial applications. It is widely used for coatings, rubbers, synthetic resins, dyes, thinners for paints and cellulose. Butanol is used as a solvent extracting essential oils, and is involved in manufacturing antibiotics and vitamins. It is also used as a flavour additive in pharmaceutical and food industries (Green 2011). Initially, butanol was produced from starch-rich products such as corn, potato, and wheat using the bacterium *C. acetobutylicum* (Jones & Woods 1986). Later however, the range of products involved in the solvent fermentation was expanded by inclusion of products such as sugar beet molasses and sugar cane, which are rich in sucrose. Clostridium bacteria used to produce acetone, butanol, and ethanol (ABE) are defined as solvent-producing bacteria, Gram-positive, rod-shaped, spore forming, and growing in anaerobic conditions (Zverlov *et al*. 2006; Lütke-Eversion & Bahl 2011). These kinds of strains are able to convert carbohydrates into solvents under anaerobic conditions, through a series of chemical reactions catalysed by a number of enzymes such as lactate dehydrogenase, pyruvate ferredoxin oxidoreductase, ferredoxin oxidoreductase NADH, NADPH ferredoxin oxidoreductase, acetate kinase, acetaldehyde dehydrogenase, etc. (Linhová 2011). The bacteria that have a specific biological activity to produce butanol from carbohydrates include *C. acetobutylicum* ATCC 824, a well-known strain, extensively studied and widely used in the ABE fermentation. Its genome consists of 3.94 Mb chromosome and megaplasmid of 192 kb, which contains most of the genes responsible for the production of solvents (Nölling *et al*. 2001; Dürre 2008). There are other strains of the same genus, such as *C. beijerinckii*, *C. butyricum*, *C. pasteurianum*, etc, which are widely used in industries. But mesophilic *C. saccharolyticum* are referred to as producing more butanol because of their ability to form butyrate (Gheshlaghi *et al*. 2009). However, 1-butanol is currently the subject of many studies due to the introduction of biofuels in recent years. It is a solvent whose physico-chemical properties are close to those of gasoline. This feature sets 1-butanol to the rank of biofuels that could replace or be added to conventional gasoline. In this perspective, the industry sector looks for renewable sources for the production of biofuels and solvents for other purposes. Our work aims at exploring cassava as an alternative raw material because of its many industrial advantages. Cassava starch and mashed cassava were tested in this study for the fermentative production of butanol using the bacterium *Clostridium acetobutylicum* DSM 1731 exhibiting good fermentative ability on the starch media.

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MATERIAL AND METHODS

Microorganisms. *Clostridium acetobutylicum* DSM 1731 was selected for this work following the preliminary screening tests performed with the strains of *C. acetobutylicum* LMG 5710 and *C. acetobutylicum* DSM 1731, the two strains with amylolytic properties. The cultures were cultivated in triplicate, with each strain in 100 ml Erlenmeyer flasks containing 50 ml of culture medium and cassava starch. Incubation took place in the anaerobic chamber (Concept 400; Ruskinn Technology Ltd., Pencoed, UK) at 37°C for 96 hours.

Spores and inoculum preparation

Spores preparation. All preparations conducted for the conservation of spores were performed in strictly aseptic and anaerobic conditions.

Spores from *C. acetobutylicum* with catalog number DSM 1731 were obtained from culture broth following the fermentation carried out in TYA medium (Shaheen *et al*. 2000). TYA medium is composed of glucose 30 g/l, tryptone 12 g/l (Fluka/Sigma-Aldrich, Switzerland), yeast extract 4 g/l (Imuna Pharm, Šarišské Michalany, Slovakia), KH_2PO_4 1 g/l (Lachema, Brno, Czech Republic), CH_3COONH_4 6 g/l (Chemos CZ, s.r.o., Czech Republic), $MgSO_4 \cdot 7H_2O$ 0.6 g/l, and $FeSO_4 \cdot 7H_2O$ 0.2 g/l (both Lachema, Brno, Czech Republic). The spores were recovered by centrifugation of the culture broth placed in sterile centrifuge tubes. The tubes were filled with ethanol and left for a period of at least 30 min before sterilisation. They were then rinsed twice with sterile distilled water. The culture broth was centrifuged for 10 min at a speed of 10 000 rpm using Sigma 1K15 centrifuge (Sigma Laborzentrifugen, Osterode am Harz, Germany). After centrifugation, the supernatant was removed, the remaining pellet was recovered and rinsed with sterile distilled water. The rinsing and centrifugation were repeated twice to remove the residual substrate. After last rinse 40 ml of sterile distilled water was added to the pellet and the tube content was thoroughly mixed. The spore suspension was then dispensed into pre-sterilised Eppendorf micro tubes using 1 ml micropipette. Eppendorf micro tubes with the spore suspension were kept and stored in a refrigerator at 4°C

Inoculum preparation. For the inoculums preparation, we performed a thermal shock of spores obtained from bacterial cells and kept previously in a cold room at 4°C for a few days. This material was heated at 80°C for 30 s in a water bath and then immediately cooled in an ice bath. The culture was transferred into the preculture medium previously sterilised (see preparation of medium). The pre-fermentation cultivation was performed in the anaerobic chamber (Concept 400; Ruskinn Technology Ltd., Pencoed, UK) at 37°C for 24 h for inoculation. The media for the actual fermentation were inoculated after between 16 and 18 h from the previous culture. The samples withdrawn from the pre-culture were observed microscopically by microscope Olympus BX51 (Olympus, Tokyo, Japan) before transferring them into the second culture stage in the volume ratio of 1 : 10. The microscopic observation was performed in a common way on microscopic slides without any staining with the use of oil-immersion lens (magnification 1000). From four culture flasks only two providing the best growth were selected.

Selection of culture media and composition

Selection of culture medium based on cassava starch. Culture media made up with different concentrations of starch were tested to select the appropriate culture medium for the production of butanol. The media were prepared from TYA medium, without tryptone, glucose having been replaced with starch in concentrations ranging from 30 g/l to 100 g/l. They were then transferred into Erlenmeyer flasks of 100 ml after adjusting the pH to 6.8 with 25% H_2SO_4.

Selection of culture medium based on mashed cassava. To obtain the mashed cassava medium, cassava roots were initially peeled and cut into small pieces before grinding. The grinding was done using a milling machine: knife mill, Grindomix GM 200 (Retsch, Haan, Germany). The milling lasted 5 min at a rotation of 7000 rpm.

Three preliminary tests were carried out using media containing 150, 175, and 200 g/l of mashed cassava in order to select the suitable quantity of cassava mash for the butanol production. Gel suspensions of mashed cassava were carefully prepared (see M2 media preparation below), and transferred into Erlenmeyer flasks of 100 ml after adjusting the pH to 6.8 with 25% H_2SO_4.

Preparation of M1 medium. Cassava starch suspensions with appropriate concentrations of 30–100 g/l were prepared with distilled water and heated to 60°C under constant stirring until complete gelatinisation, and then were cooled to room temperature. Nutritive substances ($MgSO_4 \cdot 7H_2O$, KH_2PO_4, CH_3COONH_4,

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Table 1. Composition of culture media made with varying concentrations of cassava starch

Components	Concentration (g/l)	Products references
Starch	30–100	from laboratory processing
Yeast extract	2.0	Oxoid, UK
$MgSO_4 \cdot 7H_2O$	0.3	Lach-Ner s.r.o., Czech Republic
KH_2PO_4	0.5	Lachema, Czech Republic
CH_3COONH_4	3.0	Chemos, Czech Republic
$FeSO_4 \cdot 7H_2O$	0.01	Lach-Ner s.r.o., Czech Republic

$FeSO_4 \cdot 7H_2O$), were dissolved in distilled water in a beaker. Yeast extract was added to the salt solution and the mixture was stirred until a homogeneous mixture was obtained. Gelatinised starch suspensions and salt solution were mixed in glass beakers which were made up with distilled water to a desired volume. After adjusting the pH to 6.8 with 25% H_2SO_4, the content was transferred into Erlenmeyer flasks. The cultures were prepared in triplicate for each concentration of starch in 250 ml Erlenmeyer flasks, each containing 100 ml of the culture medium. These were then sterilised at 120°C for 15 min in an autoclave (Chirana, Prague, Czech Republic) and cooled to room temperature before being inoculated, and then were incubated in an anaerobic chamber (Concept 400) at 37°C for 120 hours. The concentrations of the components such as nutrients (yeast extract, $MgSO_4 \cdot 7H_2O$, KH_2PO_4, CH_3COONH_4, $FeSO_4 \cdot 7H_2O$), remained unchanged in all tests.

Preparation of M2 medium. A suspension of 175.0 g of fresh cassava (25–30% dry weight) was ground in a mixer after peeling. Thereto was added 500 ml of distilled water before heating the content to 60°C under constant stirring until complete gelatinisation. The content was cooled to room temperature and transferred into a measuring cylinder. Then the volume was made up water to the desired level (1 l) with distilled water. The pH of the gelatinised solution was then adjusted to 6.8 before sterilisation. The culture media MCSL1, MCSL2, MYE1, and MYE2 were prepared using the same methods as those for media M1 and M2 for which nutrients such as corn steep liquor (CSL), yeast extract (YE), and minerals were added.

For the preparation of M2 media, mashed fresh cassava (with 25–30% starch, dry weight) was mixed with 500 ml of distilled water. The suspension was heated to 60°C under constant stirring until completely gelatinased. The content was cooled to room temperature and transferred into a measuring cylinder. Then the volume was made up to the required level (1 l) with distilled water. After adjusting the pH to 6.8 with 25% H_2SO_4, the gel suspension (medium) was transferred into 250 ml Erlenmeyer flasks in triplicate for each concentration, each containing 100 ml of the culture medium. Sterilisation, inoculation, and incubation of the culture media were carried out as in the previous case of M1 medium.

Culture media MCSL1, MCSL2 and MYE1, MYE2 were M1 and M2 media improved with external nutrients such as corn steep liquor (CSL), yeast extract (YE) and inorganic salts (Tables 1 and 2). All cultures were prepared in triplicate in 250 ml Erlenmeyer flasks, each containing 100 ml of the appropriate culture

Table 2. Composition of culture media containing cassava starch and cassava mash enriched with corn steep liquor and yeast extract

Components	Medium composition (g/l)					
	M1	M2	MCSL1	MCSL2	MYE1	MYE2
Cassava mash	–	175	–	175	–	175
Starch	70	–	70	–	70	–
Yeast extract	2.0	–	–	–	2.0	2.0
Corn steep	5.0	–	5.0	5.0	5.0	5.0
$MgSO_4 \cdot 7H_2O$	0.3	–	0.3	0.3	0.3	0.3
KH_2PO_4	0.5	–	0.5	0.5	0.5	0.5
CH_3COONH_4	3.0	–	3.0	3.0	3.0	3.0
$FeSO_4 \cdot 7H_2O$	0.01	–	0.01	0.01	0.01	0.01

M1 – Cassava starch medium; M2 – mashed cassava medium; MCSL1, MCSL2 – M1 or M2 + with corn steep; MYE1, MYE2 – M1 or M2 + corn steep + yeast extract

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medium, with subsequent incubation in an anaerobic chamber (Concept 400) at 37°C for 120 hours.

Determination of substrate and products by HPLC

Preparation of samples for HPLC analysis. The samples were taken every 24 h during the experiments and centrifuged at the rotation speed 10 000 min^{-1} (8944 *g*) for 10 min in a centrifuge Sigma 1K15 (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). The supernatant was filtered through microfilters with the pore diameter of 0.2 µm. 2–3 ml of the filtrate was pipetted into vials and further analysed (HPLC analysis). The supernatant of each sample was analysed by HPLC (Agilent 1200 series; Agilent, St. Clara, USA) with a polymer column H^+ IEX form (8 µm, 250 × 8 mm; Watrex, Prague, Czech Republic). Sulphuric acid in demineralised water (*c* = 5 mmol/l) was used as the mobile phase, the flow rate was 0.5 ml/min, column temperature was 25°C, with refractive index detection and automated sample injection of 20 µl. The concentrations of glucose, lactate, acetate, acetoin, ethanol, acetone, butyrate, 2-propanol, and 1-butanol were determined.

Determination of residual starch concentration as reducing sugars. The concentration of the residual starch was determined based on the concentrations of reducing sugars whose assays were carried out using the Luff-Schoorl method (Davídek *et al*. 1977)

RESULTS AND DISCUSSIONS

The first objective of this study was to find the strain of *Clostridium* possessing a high capacity to produce higher amounts of butanol using media based on cassava. *Clostridium acetobutylicum* DSM 1731 was selected for further work due to its ability to convert starch into butanol. At 96 h fermentation, it produced 17.8 g/l of solvents, i.e. 12.3 g/l of butanol, 3.7 g/l of acetone, and 1.8 g/l ethanol, whereas *Clostridium acetobutylicum* LMG 5710 produced 11.5 g/l of the total solvent, including 8.2 g/l of butanol, 2.5 g/l of acetone, and 0.8 g/l ethanol. Based on this result, all fermentations conducted in the course of this work were carried out using the strain *C. acetobutylicum* DSM 1731.

The second objective of this study was to evaluate and optimise the production of solvents considering to the initial concentrations of starch and mashed cassava in the culture media. The results showed that the production of solvents in the starch media increased with the increasing initial starch concentration. This result is consistent with that obtained by the investigation on *C. saccharoperbutylacetonicum* N1-4 (ATCC 13564) using sago starch by (Al-Shorgani *et al*. 2011). However, it turned out that the production of butanol and ethanol began to slow down significantly in such environments in which the initial starch concentration exceed 60 g/l. As for acetone, the decline began in the media containing 80 g/l of the initial starch (Figure 1).

The comparison of the products resulting from the fermentation done in various culture media for 96 h indicated that the culture medium containing 70 g/l as the initial starch concentration yielded maximum concentration of solvents (17.8 g/l), i.e. 12.3 g/l 1-butanol, 3.7 g/l acetone, and 1.8 g/l ethanol. The optimum production of butanol, 12.3 g/l in 96 h, corresponds to a yield of 25% and 0.13 g/l/h productivity. However, these values are low compared to 31% and 0.28 g/l/h obtained by (Li *et al*. 2014) in the work on a mutant of *C. acetobutylicum*. Certainly, the concentration of 12.3 g/l of butanol was lower with respect to the initial starch concentration (70 g/l)

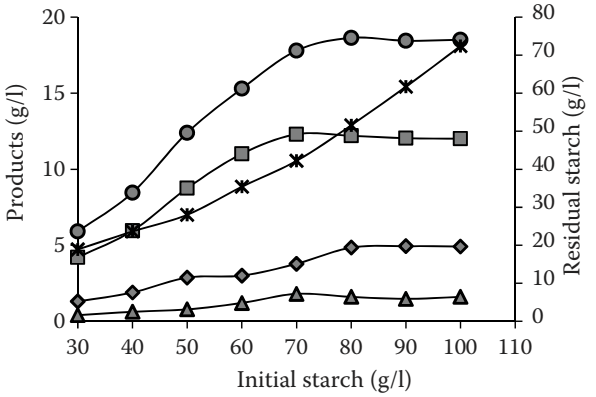

Figure 1. Effect of the initial concentration of starch on the solvent and residual starch concentrations during the acetone, butanol, and ethanol (ABE) fermentation by *C. acetobutylicum*

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and also to our expectations, but it approximates the results obtained in the industrial context where the concentration of butanol hardly exceeds 13 g/l (Ramey & Shang-Tian 2004). This is corroborated by Al-Shorgani *et al*. (2012), whose work on the production of butanol from butyric acid by *C. saccharoperbutylacetonicum* N1-4 (ATCC 13564) refers to 12.9 g/l of butanol. On the other hand, the limitation in the butanol production is related to its strong inhibitory effect (Ramey & Shang-Tian 2004). The bacterial strain used in this study has a dual role. *C. acetobutylicum* DSM 1731 firstly ensures the hydrolysis of starch into glucose, which is then converted into other products. The success in the starch hydrolysis and the efficiency of the glucose converting depend on the enzyme activity of bacterial cells. Therefore, the high levels of the residual starch which are observed at the end of fermentation explain the effectiveness level of the bacterial enzymes that are involved in the conversion of starch to biosolvents. The enzymes α-amylase and glucoamylase, which are mainly involved in the digestion of starch, are produced by bacterial cells, but their secretion is limited in time and affects the extension of hydrolysis. As an example, the studies on *C. acetobutylicum* DSM 13864 showed that the activities of α-amylase and glucoamylase secreted in the culture broth reached their maximum between 12 and 16 h after the broth inoculation (Liew *et al*. 2006). The pH is another factor that affects the smooth running of the starch hydrolysis. The changes in pH can cause incomplete digestion of starch after the termination of the enzymatic activity of α-amylase, this enzyme being destroyed by acidolysin, an extracellular protease active when pH decreases to 5.2 (Liew *et al*. 2006). The optimal pH for the fermentation of *Clostridium* is between 6.5 and 6.8. The drop in pH due to the acidification of the culture medium creates a harsh environment for the cells.

Regarding the formation of acids, it can be concluded from the results that M2 medium can be regarded as a poor medium for the production of acids when comparing the acid output in media M1 and M2. The deficit in the production of acids in M2 medium may be explained by the fact that it is composed solely of water and cassava which is poor in nitrogen compounds, in particular proteins, and in lipids. Probably, the lack of nitrogen substituents and lipids can initiate a low production of acids since proteins and volatile fatty acids (acetate and butyrate) are essential for the expression of enzymes associated with acetogenesis and solventogenesis ABE fermentation (Chen & Blaschek 1999). This hypothesis is corroborated by the work of Yang *et al*. (2009), who reported that proteins and volatile fatty acids also play an important role in increasing the production of cassava solvents. The culture results in M2 medium indicated the butanol production of 11.4 g/l, this being lower than 12.3 g/l obtained in medium M1. But industrially, M2 medium offers three advantages, namely: the optimal use of all the starch of cassava tubers, thus the reduction of input costs; culture in this medium does not require the addition of external nutrients such as peptone, yeast extract, and mineral salts. Then the draff resulting from this fermentation can be used as a feed for livestock. On the other hand, microscopic observations (Olympus BX51; Olympus, Tokyo, Japan), of samples of M1 and M2 cells did not provide evidence or distinctive signs of the morphological changes in the cells of the two samples. All cells were very similar in shape and also in the development of spores. According to our observations, sporulation begins from the 48th hour. Spores development in the cells before being released into the broth begins from the 72th hour. At this point, the production of solvents extensively increases, especially that of butanol and acetone (Table 3). The emergence of spores in solvent producing anaerobes bacteria is an important indicator of ABE fermentation, since the *Clostridium acetobutylicum Spo0A* gene encodes both as the regulator of the transcription of the sporulation and solvent formation (Jones *et al*. 2011). On the other side, fermentation carried out in the culture medium M2 showed a high viability of the culture medium neatly prepared from the whole cassava. This study confirmed the non-toxic nature of cassava (Gunorubon 2012), and proved that cassava tubers may be a desired feedstock for large-scale fermentation. However, there is a distinction in the production of products. The concentrations of solvents and acids differ significantly between the culture media. The comparison of the amounts of solvents produced in the culture media M1 and M2 shows that *C. acetobutylicum* DSM 1731 generated in culture medium M1 about 17.8 g/l of solvents comprising 12.3 g/l butanol, 3.7 g/l acetone and 1.8 g/l ethanol, whereas the production of solvents in culture medium M2 was 11.5 g/l butanol, 3 g/l of acetone, 1.8 g/l of ethanol, i.e. totally 16.2 g/l solvents (Table 3). It was also noted that maximum total acid concentration, 3.8 g/l, produced in medium M1, was three times as

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Table 3. Products (g/l) of the fermentation of *C. acetobutylicum* DSM 1731 performed in media M1 and M2

Times/h	Acid (g/l)				Solvents (g/l)			
	lactic	acetic	butyric	∑acids	ethanol	acetone	butanol	∑ABE
Medium M1								
24	0.18 ± 0.04	1.52 ± 0.01	1.4 ± 0.03	3.1 ± 0.02	0.4 ± 0.052	1.8 ± 0.052	5.56 ± 0.05	7.1 ± 0.18
48	0.29 ± 0.05	1.71 ± 0.02	1.8 ± 0.05	3.8 ± 0.05	0.74 ± 0.02	2.6 ± 0.013	7.4 ± 0.02	10.7 ± 0.10
72	0.44 ± 0.02	1.9 ± 0.007	1.4 ± 0.02	3.7 ± 0.00	0.81 ± 0.02	4.8 ± 0.026	11.5 ± 0.07	17.1 ± 0.07
96	0.6 ± 0.002	1.9 ± 0.002	1.3 ± 0.13	3.8 ± 0.02	1.8 ± 0.01	4.3 ± 0.052	12.3 ± 0.13	18.4 ± 0.05
120	0.6 ± 0.002	1.9 ± 0.002	1.3 ± 0.02	3.8 ± 0.05	1.8 ± 0.052	3.8 ± 0.026	11.9 ± 0.07	17.4 ± 0.07
Medium M2								
24	0.3 ± 0.007	0.8 ± 0.01	1.1 ± 0.03	2.2 ± 0.13	0.7 ± 0.05	0.9 ± 0.04	2.5 ± 0.05	4.32 ± 0.2
48	0.95 ± 0.01	0.51 ± 0.02	0.1 ± 0.05	1.5 ± 0.02	1.03 ± 0.02	1.8 ± 0.002	5.5 ± 0.02	8.46 ± 0.23
72	0.56 ± 0.00	0.6 ± 0.007	< 0.1	1.2 ± 0.02	1.35 ± 0.02	2.2 ± 0.09	6.4 ± 0.14	11.8 ± 0.14
96	0.44 ± 0.00	0.5 ± 0.002	< 0.1	0.9 ± 0.00	1.8 ± 0.01	3.0 ± 0.10	11.4 ± 0.1	16.2 ± 0.3
120	0.3 ± 0.04	0.56 ± 0.00	< 0.1	0.8 ± 0.00	1.8 ± 0.007	3.1 ± 0.05	11.6 ± 0.00	16.4 ± 0.03

Values are the means of three replicates with standard deviations (±) of the calculated concentrations

high as maximum total acid concentration of 1.2 g/l reached in medium M2 (Table 3). The concentrations of lactic and acetic acids obtained at the end of the fermentation in culture medium M2 were, respectively, one half and one third of those obtained in medium M1, while the production of butyric acid in medium M2 decreased drastically to a concentration bellow 0.1 g/l after 48 h of fermentation. In addition, the effect was followed in this study of the nutrients on the production of solvents (acetone, butanol, ethanol) and acids (lactic, acetic, butyric) and the results are summarised in Table 4. Even when the culture medium M2 was not supplemented by any nutrients, *C. acetobutylicum* DSM 1731 was able to produce 16.2 g/l solvents. This amount of solvents is lower than 17.4 g/l of solvents obtained in culture medium M1. The major loss of solvents refers to butanol, whose production was reduced to 11.4 g/l in medium M2 as compared with 12.2 g/l, produced in medium M1 (Table 4).

On the one hand, the addition of nutrients to culture media MCSL2 and MYE2 has resulted in the decrease of the solvents production. When comparing the concentrations of solvent determined in culture media MCSL2 and MYE2 after fermentation to those obtained in medium M2, we can see a strong decrease in the production of butanol. The production of butanol decreased to 8.7 g/l and 9.2 g/l, respectively, in culture media MCSL2 and MYE2 while in medium M2 the achieved concentration of butanol was 11.4 g/l. It could be summarised that culture medium M2 fortified with inorganic salts combined

Table 4. Effects of organic and inorganic substances in the production of acids and solvents at 96 h of fermentation carried out with *Clostridium acetobutylicum* DSM 1731

Media	Acid (g/l)				Solvents (g/l)			
	lactic	acetic	butyric	∑acids	acetone	butanol	ethanol	∑ABE
M1	0.3 ± 0.00	1.9 ± 0.0	1.6 ± 0.04	3.8 ± 0.04	3.4 ± 0.04	12.2 ± 0.04	1.7 ± 0.52	17.3 ± 0.08
MCS1	0.5 ± 0.04	1.8 ± 0.0	1.7 ± 0.04	4.0 ± 0.04	2.5 ± 0.04	6.4 ± 0.52	0.5 ± 0.08	9.4 ± 0.04
MYE1	0.4 ± 0.04	2.0 ± 0.04	1.4 ± 0.08	3.8 ± 0.08	3.6 ± 0.08	12.2 ± 0.0	1.6 ± 0.01	17.4 ± 0.04
M2	0.2 ± 0.04	0.6 ± 0.0	0.07 ± 0.0	0.87 ± 0.0	3.0 ± 0.0	11.4 ± 0.24	1.8 ± 0.05	16.2 ± 0.08
MCS2	0.3 ± 0.08	1.6 ± 0.2	1.1 ± 0.0	3.0 ± 0.04	4.0 ± 0.33	8.7 ± 0.08	1.5 ± 0.04	14.2 ± 0.04
MYE2	0.4 ± 0.04	2.1 ± 0.0	1.5 ± 0.08	4.0 ± 0.63	3.7 ± 0.92	9.3 ± 0.08	1.7 ± 0.02	14.7 ± 0.53

M1 – Cassava starch medium; M2 – mashed cassava medium; MCSL1 and MCSL2 – (M1 and M2) + with corn steep; MYE1 and MYE2 – (M1 and M2) + corn steep + yeast extract; values are the means of three replicates with standard deviation (±) of the calculated concentrations

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with corn steep liquor or yeast extract did not favour the production of solvents by *C. acetobutylicum* DSM 1731. The results in Table 4 show that the addition of nutrients influenced the production effectiveness of *C. acetobutylicum* DSM 1731. On the other hand, the addition of nutrients to culture medium M1 shows that the fermentation performed by *C. acetobutylicum* DSM 1731 in culture medium MYE1 was more effective than that carried out in culture medium MCSL1.

Looking at the results of the production of acids, it can be seen in Table 4 that the culture in M2 medium proved lower concentrations of organic acid produced by *C. acetobutylicum* DSM 1731 (i.e. 0.87 g/l total acids). However, the total acid concentration from 3 g/l to 4 g/l was obtained in such culture media which had been enriched.

CONCLUSION

The results of the trials which were carried out with culture media made with different concentrations of starch indicate that the initial concentration of 70 g/l starch was found suitable for *C. acetobutylicum* DSM 1731 for maximum production of butanol, while 96 h are considered as optimal fermentation time. It can be concluded that mashed fresh cassava can be used directly as a complete culture medium without the addition of inorganic and organic nutrients as compared to media made from pure starch in which these nutrients are essential. In economic terms, M2 medium seems to be interesting for the use in industry by reducing the production costs through eliminating the supply of nutrients, mainly mineral salts and organic products. In addition, the residues of the fermentation products can be used as animal feed. As to the effect of organic substances in M1 medium, yeast extract was found more suitable for improving the culture medium as compared to the corn steep liquor.

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