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Fermentative Production of *nata-de-cashew* from Calcium Alginate Incorporated Cashew Apple Juice Medium

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Abstract

Nata produced by *Gluconacetobacter xylinum* is a pure form of cellulose without lignin and hemicelluloses unlike plant cellulose. The production of bacterial cellulose or *nata* is receiving great attention due to its wide range of application both in food and pharmaceutical industries. However, the yield is comparatively very low and the commercially used carbon sources such as glucose are relatively expensive. In this study, a pilot scale bacterial cellulose production was carried out in a 5 L capacity model jar fermentor using *G. oboediens* (*sju*-1) Acc. No. KF164613 in calcium alginate incorporated cashew apple juice (CAJ) medium. Fermentation kinetics of bacterial cellulose production was evaluated during the course of reaction. The population increase with a gradual decrease in pH was noticed up to 16th day of inoculation. Initially the population was 0.002 × 10^{11}cfu mL^{-1}, which increased to 13.24 and 25.21 × 10^{11} cfu mL^{-1} on 8th and 10th day, respectively. By 16th day of incubation, the population reached the maximum of 34.12 × 10^{11} cfu ml^{-1} and the pH of the medium dropped to 4.5. Bacterial cellulose produced per 100 g of the substrate (Yp/s) was 1.62 ± 0.01g, while it was found to be 1.30 ± 1.11g. 100g^{-1} of cells (Yp/x). With respect to total sugars, the initial concentration of 18.00 g.L^{-1} dropped to 16.14, 10.88 and 9.14 g.L^{-1} on 2nd, 6th and 8th day, respectively. On the 16th day when the population of bacterial cells was at the maximum, the total sugar content dropped drastically to 2.18 g L^{-1}. Due to the incorporation of calcium alginate in medium, the yield of *nata-de-cashew* increased and the mass production was found to be easier compared to the flask culture. Hence, it is concluded that, addition of calcium alginate to CAJ medium under static condition, inoculated with *G. oboediens* (*sju*-1) NCBI GenBank Acc. No. KF164613 in a modified jar fermentor could increase the production of *nata*.

Keywords: *Gluconacetobacter oboediens*, Cashew apple juice medium, Calcium alginate, *nata-de-cashew*, Static jar fermentor

Gluconacetobacter xylinum is usually cultured under static fermentation in a chemically undefined medium called HS medium (Hestrin and Schramm, 1954). Addition of supplements to the cell culture medium like endoglucanase, pyruvate, ethanol, lactate and sodium alginate would boost the *nata* or bacterial cellulose production (Hungund *et al.* 2013). Anusuya *et al.* (2020) had reported that *Acetobacter senegalensis* MA1 produces bacterial cellulose of about 3.6 g in Hestrin and Schramn medium.

The bacterial cellulose production by *A. xylinum* NUST 4.1 carried out by adding sodium alginate (NaAlg) into the medium significantly enhanced the yield and modified the crystalline properties of cellulose. Various water soluble polymers added into the medium were known to interfere with the

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aggregation of microfibrils into a normal ribbon assembly. Addition of water-soluble polymers such as xanthan, agar, polyacrylamide-co-acylic acid (PCA) and acetan can increase relative viscosity of the broth to reduce shear stress, hinder coagulation of bacterial cellulose during the cultivation to form uniform smaller pellets, which were found to be advantageous to transfer nutrients and oxygen into bacterial cells located inside and on the surface of the cellulose matrix (Zhou *et al.* 2007). The yield of *nata* not only depends on the composition of culture medium, but also relies upon the concentration, surface, volume, pH, aeration of the medium, cultivation method, reactor type and temperature (Chawla *et al.* 2009). The most common bacterial cellulose synthesis method is static culture, which could produce cellulose in the form of pellicles on air / liquid interface. Quite a number of studies demonstrated improvements in bacterial cellulose production under dynamic conditions, using different devices such as static tray fermentor, rotating disk, air-lift, agitated and shaking reactors (Hornung *et al.* 2007). Improved fermentation processes are required for a breakthrough towards an economical process for bacterial cellulose production. Lin *et al.* (2016) has reported that bacterial cellulose film, hydroxyl-propyl-methyl cellulose modified bacterial cellulose and dried fabricated biofilm were found to be suitable candidates for applications in delayed and rapid drug release type biofilms in pharmaceutical applications. Hence, in the present research, a modified low cost jar fermentor was designed to improve the yield of *nata-de-cashew* for various applications.

Carvalho *et al.* (2007) have developed a blended beverage, based on coconut water, cashew apple juice and caffeine for bacterial cellulose production. Kurosumi *et al.* (2009) developed an effective culture method and investigated for the possibility of producing bacterial cellulose from orange, pineapple, apple, Japanese pear and grape fruit juices by *Acetobacter xylinum* NBRC 13693.

Costa *et al.* (2009) have developed fruit powders from cashew apple and guava (*Psidium guajava* L.) residues after extracting of the juice and used as a source of vitamin C and lipid, respectively. However, cashew apple-based products are not commercially popular in India, unlike in Brazil and other countries. Cashew apple contains tannins, causing astringency, which needs to be precipitated out before making juice. According to the report of Tigressa *et al.* (2008) only 12.0 $g.100g^{-1}$ of the total peduncle is processed. Honorato *et al.* (2007) have illustrated that cashew apple juice has been used for the production of surfactants, alcoholic beverages and syrup. However, mostly the pseudo fruit is underutilized and often fed to cattle and pigs. Being a rich source of sugar, cashew apple after extraction of nut could be a cost effective culture medium for the production of bacterial cellulose in static fermentor. Hence, this research work was carried out to explore the possibility of bacterial cellulose production from calcium alginate incorporated cashew apple juice medium by static fermentation, in a modified jar fermentor using an efficient cellulose producing bacterial strain isolated from sugarcane juice.

MATERIAL AND METHODS

Starter culture and inoculum preparation

The starter culture namely, *Gluconacetobacter oboediens* (sju-1) NCBI GenBank database accession number KF164613 used in this study, was previously isolated from fermenting sugarcane juice and identified at the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, India.

Gluconacetobacter oboediens (*sju*-1) Acc. No. KF164613 was cultured in Hestrin Schramn medium. Cashew apple juice with total soluble solids (TSS) of 25°Brix was prepared and preserved at 4°C. A modified cashew apple juice (CAJ) medium containing cashew apple juice, fructose, yeast extract, citric acid and disodium hydrogen phosphate were pre-optimized to standardize the growth medium. Various components like agar, agarose, acetan, calcium alginate, chitin, chitosan, cellulose, carboxy methyl cellulose, dextran, gelatin, gellan, levan, lignosulfonate and poly ethylene glycol were taken at 0.1% level in separate 250 mL Erlenmeyer flasks and

added with other components of pre-standardised medium, sterilized and inoculated with 24 h old culture of *G. oboediens* (*sju*-1) Acc. No. KF164613 containing 20 × 10^8 cfu mL^{-1} and incubated at 31°C. Based on the yield of bacterial cellulose in the pre-optimized media (Cashew apple juice (1000 mL), 4.0 g fructose, 6.0 g yeast extract, 1.5 g citric acid, 0.5 g disodium hydrogen phosphate, 0.1%, calcium alginate, pH 6.5) was taken for further study. Mother culture was also prepared using the standardized medium (Plate 1).

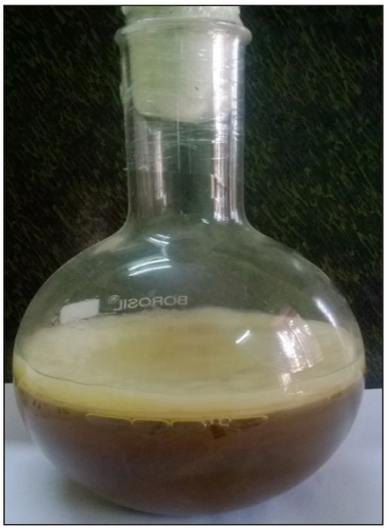

Plate 1: Mother culture of *G. obediens* in cashew apple juice medium

Fermentation in a static jar fermentor

A schematically designed (Fig. 1) 5.0 L capacity TPX™ poly-methyl-pentene transparent light weight autoclavable jar with handle was taken for laboratory scale production of *nata-de-cashew* under static fermentation during study. The lid was designed using GI sheet with four holes (4 cm) placed, at an equal distance and a handle at the centre. The holes were plugged with cotton for aeration. About 3.5 L of cashew apple juice medium was taken and closed with the lid. The entire set up was sterilized by autoclaving. After sterilization, 350 mL of seed culture was inoculated into it and incubated statically at 31°C for 20 days. Sample can be withdrawn through the holes with a sterile pipette for quantitative and qualitative analyses.

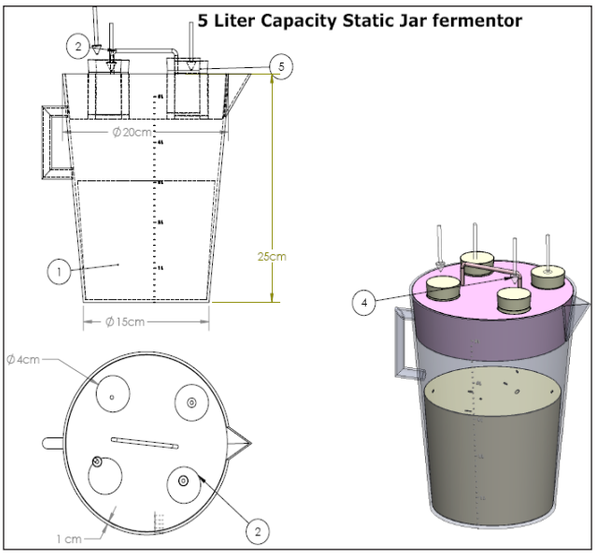

Fig. 1: Schematic design of laboratory scale static jar fermentor for the production of *nata-de-cashew*

Fermentation kinetics in Cashew Apple Juice medium

The growth kinetics of *G. oboediens* (*sju*-1) Acc. No. KF164613 in cashew apple juice medium under static fermentation in the pre-designed model fermentor was expressed in terms of growth rate (k.g $L^{-1}d^{-1}$), specific growth rate (μ.h^{-1}), C max = maximum *nata* concentration (g.L^{-1}), Qp = Nata produced (g.L^{-1}. h^{-1}), Yp/s = *Nata* produced (g.100 $g^{-1}$of sugar source used in the medium and Yp/x = *Nata* produced (g.100 g^{-1} of cells). Samples were withdrawn at two days interval up to 20 days of fermentation to quantify the changes in viable cell number, pH and total sugars following the methods suggested by (Keshk, 2014).

RESULTS AND DISCUSSION

The results of the fermentation kinetics of *nata-de-cashew* production under static fermentor using CAJ medium is presented in Table 1. The population increase from 0.002 to 34.12 × 10^{11} cfu mL^{-1} with a gradual decrease in pH (4.5) was noticed up to 16th day of inoculation. Thereafter the population reached its stationary phase (31.22 and 33.14 × 10^{11} cfu mL^{-1} on

18th and 20th day, respectively). On 20th day the pH became very much acidic recording a value of 3.5. The initial concentration of total sugars was 18.00 g L^{-1} (Fig. 2), which dropped to 16.14, 10.88 and 9.14 g L^{-1} on 2nd, 6th and 8th day, respectively. Interestingly, on the 16th day when the bacterial population was at its maximum, the total sugar content dropped to 2.18 g L^{-1} which further decreased to1.50 g L^{-1} at the end of the experiment (*i.e.*, on 20th day). It was also observed in the present study that, wide mouthed user friendly jar type model fermentor could give economical yield of *nata-de-cashew* using, *G. oboediens* strain *sju*-1 (NCBI Acc. No. KF164613).

Table 1: Fermentation kinetics of *nata-de-cashew* production using CAJ medium

Parameters	Observed values
Growth rate (K) (g. L^{-1}. d^{-1})	0.0079 ± 0.11
Specific growth rate (μ) (h^{-1})	0.0031 ± 0.04
Maximum Nata (C max) (g.L^{-1})	16.22 ± 0.17
Nata production rate (Qp) (g. L^{-1}. h^{-1})	0.27 ±0.10
Nata produced (Yp/s) (g.100g^{-1} of substrate)	1.62 ± 0.01
Nata produced (Yp/x) (g.100g^{-1} of cells)	1.30 ±1.11

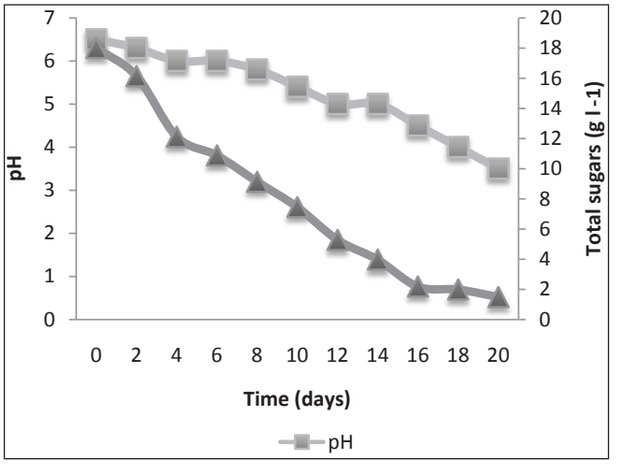

Fig. 2: Changes in pH and total sugars of CAJ medium during fermentation

Hornung *et al*. (2006) have explained the mass transfer reactions during the growth of *A. xylinum.* The cellulose growth rate ceased after about 15 days, but remnant glucose was found to be diffused through the layer and continued to be consumed, even after the stagnation of product formation, especially used for cell maintenance. After a brief lag phase, the number of cells, which produced *nata-de-cashew* increased exponentially reaching the maximum value of 8.3 × 10^9 cells and remained constant on the maximum value between days 8 to 10 during incubation. From 11th day onwards the number of *nata-de-cashew* producing cells decreased due to the cessation of cellulose production. The results obtained from the present study paralleled with the above findings. Ishida *et al.* (2003) established the use of negatively charged water-soluble cellulose derivative, carboxymethylcellulose (CMC), agar and sodium alginate to enhance *nata-de-cashew* production in static culture. This study indicated that calcium alginate incorporation in to the production medium increased the yield of cellulose to 17.8 ± 0.38 g L^{-1}, whereas, previous studies involving cashew apple juice medium without incorporation of calcium alginate had given only 14.1±0.35 g L^{-1} of *nata-de-cashew* during static fermentation.

Jessica and Ari (2017) had reported that *nata-de-coco* produced without Ammonium sulphate is able to meet good the sensory values than the *nata-de-coco* produced with Ammonium sulphate. After 8 days of fermentation, 1.2 to 1.3 cm thickness of *nata* had formed with acidic pH of 4-5. In the present study similar type of thickness had been obtained with pH reduction to 4.5 during the course of fermentation.

Pal *et al.* (2019) had developed and standardized a ready to eat, dessert food, *Nata-de-mango* from mango pulp of 22°Brix which had been incorporated into beverages upto 25% for high organoleptic acceptability. Similarly, the results of the present research uses cashew apple juice, with total soluble solids of 25° Brix to produce *nata-de-cashew* would better serve as an adjuvants in the preparation of soft drinks.

Hence, an addition of polysaccharide like calcium alginate to the production medium prepared with

underutilized sugar rich fruit juice like that of cashew apple is suggested for substantially increased *nata-de-cashew* production in bioreactors.

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