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Optimized biosynthesis of xanthan via effective valorization of orange peels using response surface methodology: A kinetic model approach

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ABSTRACT

Herein, an enhanced green production of xanthan gum has been achieved by utilizing orange peels. Response surface methodology and kinetic modeling were adapted for the process optimization and its influence on scale up production respectively. Optimal conditions for the maximum xanthan production were 1.62% acid hydrolysis, 85% carbon source of orange peel hydrolysate and 30.4 °C temperature. Furthermore, the optimized treatment was conducted in the batch culture fermentor to observe the associated variations during scale up process. In bio-fermentor, to the first time ever, xanthan production along with reducing sugar conversion and utilization rates reached 30.19 g/L, 69.29% and 99.99%, respectively. Employed characterization techniques of FTIR, XRD and HPLC confirmed the fermented product as xanthan gum and obtained an average molecular weight of 1.01×10^6 g/mol. This work on account of optimized process parameters presented maximum xanthan production from a waste material.

1. Introduction

Xanthan gum is the first bacterial-hetero-polysaccharide, synthesized by aerobic fermentation of sugars by *Xanthamonas campestris* (Moffat, Morris, Al-Assaf, & Gunning, 2016). Its production is mainly carried out in conventional stirred tank fermenters (Papagianni et al., 2001). Many factors such as temperature, pH, aeration, agitation, substrate, carbon and nitrogen sources determine the production, quality, composition and molecular mass of xanthan gum (García-Ochoa, Santos, Casas, & Gomez, 2000; Palaniraj & Jayaraman, 2011). However, industrial xanthan production often uses glucose and sucrose as a substrate, and these substrates are highly productive in delivering qualitatively good product. Unfortunately, annual escalation in substrate price, glucose and sucrose, due to higher demand raise concerns for economical fulfillment of xanthan requirement (de Jesus Assis et al., 2014). The annual production of xanthan is approximately 30,000 tons, whereas its consumption is on gradual increase annually. Recently reported price of xanthan gum ranges US$ 4000–5000/ton due to higher production costs of glucose and sucrose being US$ 400–600/ton (Li et al., 2016). Therefore, there exists a dire need to find an inexpensive alternative fermentation medium for xanthan production that may reduce its current higher production cost.

In order to economize the process indirect sources of required substrate, such as hydrolyzed rice, barley, corn flour, coconut juice and sugar cane, have been proven as successful approach (Palaniraj & Jayaraman, 2011). Nowadays, the commercialized production of xanthan has been centered to the use of inexpensive substrates, for instance, sugarcane molasses, whey (Silva et al., 2009), crude glycerol (Wang, Wu, Zhu, & Zhan, 2016), kitchen waste (Li et al., 2016), olive mill wastewaters (Lopez, Moreno, & Ramos-Cormenzana, 2001), tapioca pulp (Gunasekar, Reshma, Treesa, Gowdhaman, & Ponnusami, 2014) etc., for moving towards economically green synthesis of xanthan gum. Additionally, for further economization of the process studies based on response surface methodology (RSM) as an optimizing tool have also been conducted for maximizing the production (de Jesus Assis et al., 2014; Silva et al., 2009).

Literature search reveals that the substrate cost has a significant impact on the fermented production of xanthan gum. Economically cheaper fermentation medium instead of an expensive selection surely renders better results in terms of cost effective xanthan production. Orange peel, though being waste, is a rich source of soluble and insoluble carbohydrates, especially the fermentable sugars. Other than

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that, China being ranked among top five orange producing countries renders as an opportunity for utilizing enormously available orange peels as a raw material for economical production of xanthan gum (Lin et al., 2013). Millions tons of orange waste are produced each year during orange juice production (8–20 million tons per year) and 50% of the orange fruit is left as waste in the form of peels. Although there is high global production of oranges, but the waste has yet no bio-conversion and value addition (Aboagye, Banadda, Kiggundu, & Kabenge, 2017). Its disposal on the ground may cause serious environmental impacts, since lignocellulosic residue in peels cause serious problems in terms of land filling waste as it may cause generation of harmful leachate compounds that can pollute the earth's surface water and groundwater. On other hand, incineration of this waste, although energy taking, also requires comparatively higher temperature for being rich in watery contents. Due to its vast availability as a bio transformable material, there lies opportunity to utilize this waste material in a more attractive, cost effective, environmental friendly cleaner manner such as xanthan production through fermentation of the carbohydrate monomers of this biomass residue (Li et al., 2016; Lin et al., 2013). Furthermore, bio-transformations of its contents into other useful products such as methane, bio-ethanol, succinic acid, pectin, D-limonene and a-terpineol have been successfully proved (Angel Siles Lopez, Li, & Thompson, 2010; Ma, Cervera, & Mejía Sánchez, 1993; Marin, Soler-Rivas, Benavente-Garcia, Castillo, & Perez-Alvarez, 2007; Widmer, Zhou, & Grohmann, 2010). Owing to uniqueness of orange peels over other waste as aforesaid, and on failing to find the desired effective fermentation task earlier, we opted it as a fermentation medium for xanthan production in current study.

The study was commenced *via* utilizing RSM as an optimization technique for effectively valorizing the substrate under effective combinatorial influence of process parameters. Three main independent variables that is acid hydrolysis (0.5–2.5%) of orange peel, carbon source (50–100%) of orange peel hydrolysate and temperature (25–35 °C) were evaluated in terms of their individual and combined effects on optimum xanthan production. The ranges of all three parameters were selected on the basis of previous studies. The temperature range of 25–35 °C was found to be promising for better xanthan production as reported by (Ben Salah et al., 2010). Acid hydrolysis ranges has been reported by (Gunasekar et al., 2014). Whereas, several studies reported carbon source ranges of sugars for xanthan production (Ben Salah et al., 2010; Papagianni et al., 2001). Furthermore, the optimized treatment with maximum xanthan production was proceeded toward batch culture fermentation in a 15 L bio-fermentor to further study its feasibility at larger scale fermentation. Additionally, batch cultivation kinetic models with a 15 L bio-fermentor were proposed for the kinetics of cell growth, product formation and substrate consumption to analyze the fermentation behavior. Finally, product characterizations were conducted for qualitative concern by subjecting the final product to spectroscopy of fourier transform infrared (FTIR) techniques, X-ray diffractometry (XRD) and high-performance liquid chromatography (HPLC).

2. Materials and methods

2.1. Microorganism and inoculum preparation

The productive strain of *Xanthomonas campestris* was purchased from the BeNa Culture Collection, Beijing, China. It was first cultivated on yeast peptone (YP) agar plates containing the medium which includes sucrose 20 g/L, yeast extract 1 g/L, peptone 5 g/L, beef extract 3 g/L with 12 g/L agar with the adjusted pH to 7.0. These plates were grown at 30 °C for 72 h. Then a loopful of cells was transferred from the YP agar plate to a 250 mL conical flask containing 50 mL of liquid YP medium with the sucrose replaced by glucose. Then flask was incubated at 30 °C with 180 rpm for 28 h.

2.2. Reagents, feedstock and orange peel hydrolysate (OPH) preparation

Commercial xanthan gum was purchased from Aladdin Industrial Corporation (Shanghai, China). The orange peel was collected from the local market of Shanghai, China. Then, orange peel was crushed by a mechanical mixer immediately after collection. In a typical hydrolytic process, 500 g orange peel was mixed with 1 L water and 0.5–2.5% sulphuric acid was added and hydrolyzed at 121 °C for 60 min.

2.3. Detoxification of orange peel hydrolysate

Followed by the hydrolysis, detoxification was carried out by using activated carbon in method described by (Khedkar, Nimbalkar, Gaikwad, Chavan, & Bankar, 2017). The pH of OPH was gradually increased by using sodium hydroxide solution and then 3% decolorizing activated carbon was added followed by shaking at 200 rpm for 2 h; further filtration was performed to remove insoluble matters containing activated carbon. Finally, the obtained OPH was used as the fermentation medium.

2.4. Characterization of orange waste

Proximate analyses of orange peel (OP) and OPH were done by applying standard methods described by (Khedkar et al., 2017). The representative composition of OP and OPH are shown in Table 1.

2.5. Culture media preparation and fermentation

OPH was used as the main carbon source with distilled water as per RSM ranges in culture media. The other medium used for xanthan gum production contained (g/L): Yeast extract, 2; K_2HPO_4, 2.5; $MgSO_4$, 0.2; citric acid, 2; H_3BO_3, 0.006, $ZnCl_2$, 0.006; $FeCl_3$, 0.0024 and $CaCO_3$, 0.02. The medium pH adjusted to 7.0 and then solutions were sterilized for 20 min at 115 °C. The shaking-flask fermentation was carried out in 250 mL conical flasks containing 50 mL of fermentation medium. The medium was inoculated with 10% (v/v) *X. campestris* culture. After that, shaking-flask cultures were incubated at 30 °C at 180 rpm for 72 h.

Batch culture in a 15 L stirred bioreactor was conducted with initial 8.0 L working volume (supplied by Shanghai Guoqiang Bioengineering Equipment Co., Ltd.). The optimized treatment with maximum xanthan gum from RSM was performed in this batch culture fermentation. The culture was incubated at 30 °C and pH of the medium was adjusted to 7.0 after sterilization. The air flow rate was adjusted at 1 vvm and the stirring rate was 300–400 rpm. During the fermentation, pH was maintained at 7.0 by adding 2.0 M NaOH.

2.6. Analytical methods

2.6.1. Determination of biomass dry cell weight (DCW)

Dry cell weight was determined by centrifugation of samples at

Table 1
Characterization of orange peel OP and OPH.

Parameters	Orange peel (OP)[a]	Orange peel hydrolysate (OPH)
Total Solids	22.07%	10.65%
Total Sugar	10.1%	58.68 mg/mL
Reducing Sugars	–	49.92 mg/mL
Protein	7.8%	–
Ash	2.81%	–
Lipid	3.63%	–
Cellulose	34.53%	–
Pectin	15.28%	–
Lignin	7.2%	–
Hemicellulose	11.38%	–

[a] The values of OP were calculated based on dry mass.