International Journal of Chemical Sciences and Applications
ISSN 0976-2590, Online ISSN 2278 – 6015
Vol 4, Issue 1, 2013, pp 73-76
http://www.bipublication.com

AMENDMENT OF BIOSLUDGE CAN ENHANCE FERULIC ACID BIOCONVERSION

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[Received-05/11/2011, Accepted-17/03/2013]

Abstract:

The effects of biosludge amendment into the culture medium during biotransformation of ferulic acid into vanillin using *Staphylococcus aureus* were studied. The study involved FA (ferulic acid) as the control and FAB (ferulic acid + biosludge) as the treatment. With HPLC analysis and product quantification, the results revealed that *S. aureus* consumed ferulic acid more quickly with biosludge amendment in the medium on day 4 with vanillin accumulation of 22.85 mg/l, whereas with ferulic acid alone as the substrate with vanillin concentration was found to be 10.33 mg/l on day 7 of incubation. The outcomes suggest promising potentials of biosludge in enhancing ferulic acid bioconversion with almost a doubled rate of bioconversion.

Keywords: Ferulic acid, *Staphylococcus aureus*, biosludge, vanillin

1. INTRODUCTION

Ferulic acid is a very significant component for the structure and the biology of cell wall as it can crosslink polysaccharide chains through dimerisation reaction [1]. Like *p*-coumaric acid, ferulic acid is a hydroxycinnamic acid occuring widely in the cell walls of graminaceous plants [2,3]. There have been many studies of exploiting microorganisms in transforming hydroxycinnamic acids to corresponding hydroxybenzoates upon the production of ferulic acid esterase enzyme. Because of having microbial origin, ferulic acid esterase has good industrial importance [4]. These benzoates serve as important components of natural flavors and fragrances and have antioxidant activities like other plant extracts [5].

A number of industrial and food applications are reported for ferulic acid, especially based on its microbial degradation to vanillin. Vanillin is a well-known name in food industries, especially those of ice-cream and chocolate. It is one of the most important aromatic flavor compounds used in foods,

beverages, perfumes and pharmaceuticals [6]**.** Considering the increasing demand of natural products, development of flavors via biotechnological processes offers an alternative to natural than chemical sources [7].

On the other hand, sewage sludge is considered to be a valuable fertilizer to soil for their plant nutrient content [8]. Biosludge enhances the microflora in nutrient deficient conditions. Extracellular polymeric substances (EPS) crucial to flocculation and dewatering of activated sludge and microstructure of methanogenic granular sludge are the metabolic products accumulating on bacterial cell surface [9-12]. EPS are composed of a wide range of organic substances with carbohydrate being a major proportion in the pure cultures [13].

This study reports the capability of *Staphylococcus aureus* to degrade ferulic acid into vanillin. Effect of biosludge as the carbon sources on the production of metabolites was the interest. The purpose of selecting biosludge as a composite source of carbon is enhancing vanillin production by *S. aureus* faster than ferulic acid alone in the media.

2. Materials and Methods

2.1. Microorganism:

S. aureus was isolated from soil on the basis of its ability to grow in ferulic acid containing medium. Pure cultures of these strains were maintained in a mixed medium containing both beef extract and peptone. Further, the population of *S. aureus* was enumerated by frequent sampling from the culture flasks on alternative days.

2.2. Substrate and treatment design:

Substrate used for vanillin production was ferulic acid. Treatments designed are as follows: (1) ferulic acid (FA) as the control and (2) ferulic acid + biosludge (FAB). Biosludge was collected from a wastewater treatment plant and sterilized at 121^{o}C for 20 min before amendment. Table 1 gives the analytical characteristics of the biosludge.

2.3. Medium and culture conditions:

4% of 5M ferulic acid and 5% w/v of biosludge were added to FA and FAG, respectively. After the growth of *S. aureus* in nutrient broth for about 5 days, 1 ml cell suspension (approximately 14 x 10^6 CFU/ml of culture) was transferred to FA and FAB in 100 ml flasks each containing 25 ml of minimal medium [14] with ferulic acid and biosludge amendments, respectively. pH of the media was adjusted to 7.2. The cultures were incubated at 35^{o}C and analyses were carried out on day-to-day basis for a period of 8 days of incubation to detect the degradation of ferulic acid. Every analysis was carried out in triplicate.

2.4. Extraction and detection of metabolites from the culture media:

For the extraction of ferulic acid and its degradation product from the culture media, culture supernatants were obtained by centrifugation. These were acidified (pH 1-2) and extracted with equal volume of ethyl acetate. The ethyl acetate was evaporated in vacuum and the residue was re-dissolved in 50% methanol. This processed culture filtrate was subjected to HPLC. HPLC analyses and quantification of ferulic acid and its degradation products were performed at 254 nm and 310 nm respectively. The compounds in sample were identified by comparison with retention times of the standard.

3. Results and Discussion

Vanillin was the major bioconversion product that was comparatively analyzed in the treatments. The amount of vanillin in FA

reached 10.33 mg/l on day 7 of incubation (Figure 1). On the other hand, FAB exhibited an increased rate of biodegradation of ferulic acid producing 22.85 mg/l of vanillin on day 4 of incubation which marks a doubled rate of bioconversion in comparison to the medium with absence of any carbon supplement (Figure 2).

Biosludge aided in the formation of high density cultures [15] of *S. aureus* with the product formation in a shorter incubation period due to composite carbon sources in it. Additionally, EPS might have played a significant role in this aspect as they form a protective layer over the bacterial cells against the harsh environmental conditions and also serve as sources of carbon and energy during starvation [9]. Biosludge may also aid in enhancing the production of certain essential primary metabolites by *S. aureus* that helped the bacteria to stabilize themselves in the medium.

Today sludge accumulation is increasing worldwide with rapid industrialization which creates many environmental issues. With these results, it can be suggested that microorganisms and their enzymes play a major role in conversion of these bioresources into valuable natural products both for commercial and socio-economic purposes.

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Table 1. Analytical characteristics of the biosludge

Parameter	Concentration
Dry matter (g/kg)	185
pH (H_2O)	6.6 ± 0.4
Electrical conductivity at 25°C (dS/m)	2.3 ± 0.3
Organic carbon (g/kg)	159 ± 5.5
Total nitrogen (g/kg)	19.2 ± 2.1

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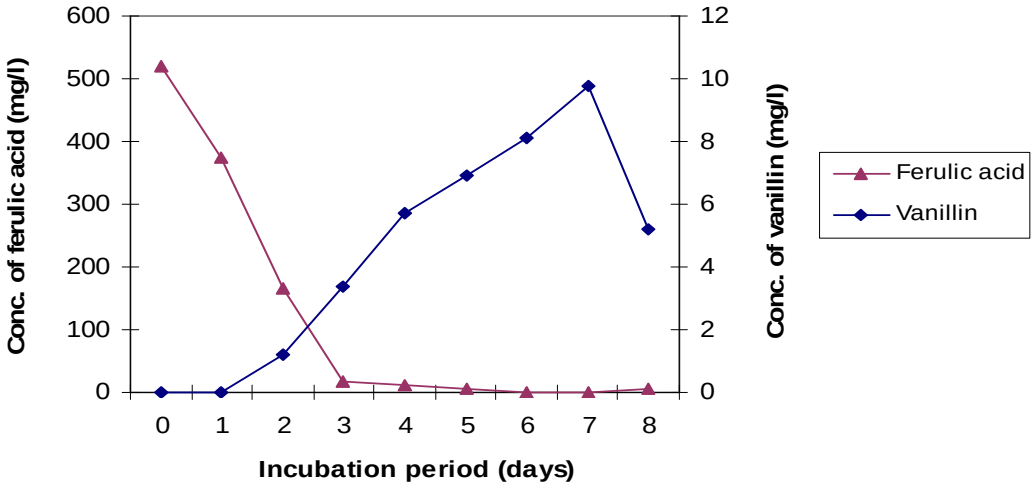

Figure 1. Concentration of ferulic acid and vanillin in treatment FA (ferulic acid) on a time-course basis.

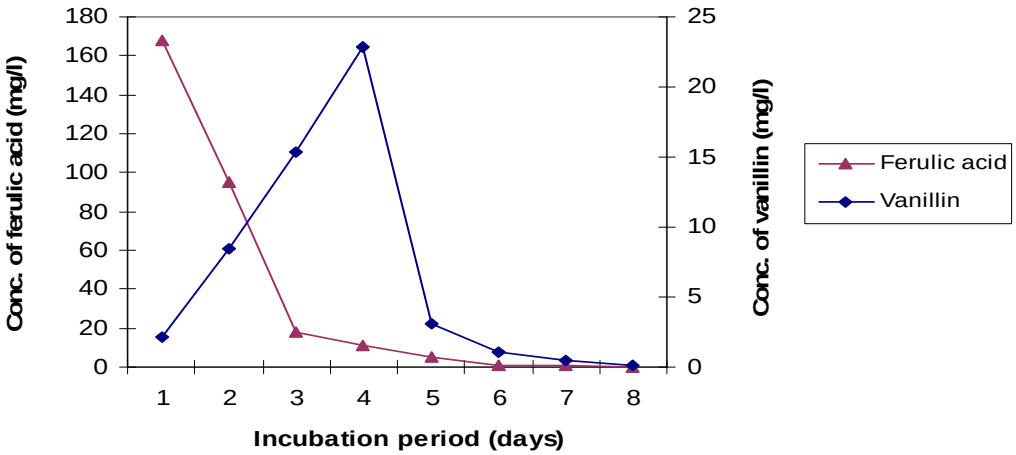

Figure 2. Concentration of ferulic acid and vanillin treatment FAB (with biosludge amendment) on a time-course basis.