Journal of Chromatography A, 1217 (2010) 1537–1542

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Capillary electrophoresis for the monitoring of carboxylic acid production by *Gluconobacter oxydans*

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ARTICLE INFO

Article history:
Received 1 September 2009
Received in revised form
22 December 2009
Accepted 24 December 2009
Available online 4 January 2010

Keywords:
Capillary electrophoresis
Organic acids
Complex forming agents in electrolyte solution
Bioprocess monitoring

ABSTRACT

Determination of carboxylic acids in *Gluconobacter oxydans* fermentations of wheat straw hydrolyzate was carried out. This matrix is of complex composition containing carbohydrates, organic compounds (e.g., amino acids, toxins), and inorganic salts making the analysis challenging even with separation techniques. A method based on capillary electrophoresis with indirect UV detection was developed for the simultaneous quantification of 18 carboxylic acids. The background electrolyte solution of ammonia, 2,3-pyridinedicarboxylic acid, and Ca^{2+} and Mg^{2+} salts, containing myristyltrimethylammonium hydroxide as a dynamic capillary coating reagent, was validated for the robust and repeatable separation of the carboxylic acids. Intraday relative standard deviations in the optimized method were less than 1.6% for migration times and between 1.0% and 5.9% for peak area. Interday relative standard deviations were less than 5.0% for migration times and between 5.7% and 9.3% for peak area. With 11 nl injected, detection limits for the analytes were between 10 and 43 μmol/l. Detection limits ranged from 0.1 to 0.5 pmol at signal-to-noise ratio of 3. The results demonstrated that wheat straw hydrolyzate was a suitable substrate for *G. oxydans* with a product yield of 45% for the formation of xylonic acid from xylose and 96% for the formation of gluconic acid from glucose.

1. Introduction

Monitoring and control of processes in bioreactors is of critical importance in biotechnology [1]. The essential tasks in bioprocess monitoring are the determination of substrate uptake rate, specific growth rate of the organisms, and the product formation rate. In practice, the most widely measured and controlled parameters are pH, dissolved oxygen and carbon dioxide, temperature and pressure [2,3]. Carboxylic acids are involved in many metabolic processes of the cell and they are important metabolites of several biochemical pathways in microorganisms. Because they are frequently either the main products or significant by-products in bioprocesses, monitoring them is often essential [4].

There has been a considerable interest in the use of lignocellulosic materials as a renewable source of chemicals for over two decades now [5]. Lignocellulose is a highly promising raw material; it is a natural and cheap polymer that is abundantly present in agricultural waste (wheat straw, corn stalks, soybean residues, sugarcane bagasse), industrial waste (pulp and paper industry), forestry residues, and municipal solid waste. Lignocellulose is estimated to account for about 50% of all biomass on Earth [6]. It consists of three main components: cellulose, hemicellulose, and lignin. Wheat straw is composed of 35–45% cellulose and 20–30% hemicelluloses with a relatively low lignin content (<20%) [7]. Cellulose and hemicellulose are composed of chains of carbohydrate molecules that can be hydrolyzed to monomeric sugars. Cellulose is composed of glucose and hemicellulose of galactose, mannose, glucose, xylose, and arabinose [8]. The pentose monosaccharide xylose together with the hexose monosaccharide glucose, are two of the most abundant sugars found in nature. Xylose is the predominant hemicellulosic sugar of hardwoods and agricultural residues, accounting for up to 25% of the dry biomass of some plant species. The abundance and ease of isolation of xylose makes it an important potential feedstock for the production of bulk chemicals such as carboxylic acids [9].

Most monomeric sugars can be metabolized by microorganisms, but an organism which is able to efficiently convert a variety of sugars (pentoses and hexoses) to useful products, and to tolerate toxins and stress conditions is required to exploit lignocellulosic material [6]. Bacterial metabolism of xylose characteristically generates multiple products, including alcohols (butanol, ethanol, isopropanol, 2,3-butanediol), carboxylic acids (acetic, butyric, formic, and lactic), polyols (arabitol, glycerol, xylitol), ketones (acetone), and gases (methane, carbon dioxide, hydrogen) [1]. *Gluconobacter oxydans* has an exceptional capacity for the oxidative

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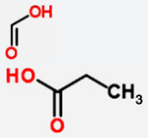
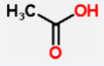
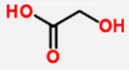
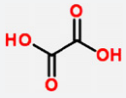
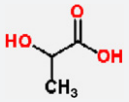
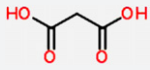
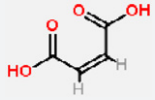
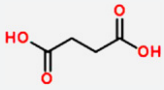
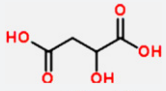
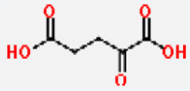
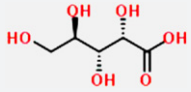
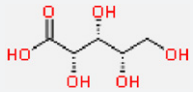
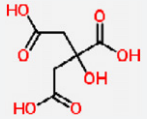
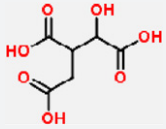
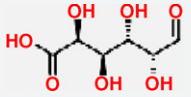
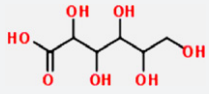
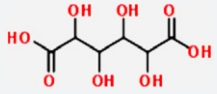
doi:10.1016/j.chroma.2009.12.075

Table 1
Carboxylic acids and their structures.

Compound	Molecular weight (g/mol)	Structure	Compound	Molecular weight (g/mol)	Structure
Formic acid	46.026		Acetic acid	60.052	
Propionic acid	74.078		Glycolic acid	76.052	
Oxalic acid	90.036		Lactic acid	90.078	
Malonic acid	104.062		Maleic acid	116.072	
Succinic acid	118.088		Malic acid	134.088	
α-Ketoglutaric acid	146.098		Arabonic acid	166.13	
Xylonic acid	166.13		Citric acid	192.124	
Isocitrate	192.124		Galacturonic acid	194.14	
Gluconic acid	196.156		Galactaric acid	210.139	

transformation of carbohydrates to polyhydric alcohols, and carboxylic acids [5].

The most widely available and accessible sources of lignocellulosic biomass are agricultural residues [10]. Wheat straw is the major crop residue in Europe and the second largest agricultural residue in the world [11]. The wheat straw hydrolyzate used here was prepared as a xylose rich hydrolyzate, containing small amounts of glucose and arabinose. *G. oxydans* is able to convert glucose and xylose to gluconic acid and xylonic acid, respectively, and these two carboxylic acids can be further used as ligands, buffers, dispersants, etc.

Traditionally, carboxylic acids have been analyzed by gas chromatography (GC) [12,13] and either by liquid chromatography (LC) [14–17] or by its submethod ion chromatography (IC) [18,19]. However, these methods have some deficiencies. When using GC, traditionally the carboxylic acids have been analyzed after derivatization to make them volatile, and therefore direct quantification has not been used [20]. LC is time-consuming and limited by a narrow linear dynamic range, high limits of detection (g/l), and susceptibility to matrix interferences [14]. IC lacks selectivity for carboxylic acids, which are weakly retained onto ion exchange materials. Furthermore, it has fairly modest chromatographic efficiency and low duration of the stationary phases [21].

Capillary electrophoresis (CE) is a versatile technique with no special need for derivatization and no limitation on polar solvents, analytes or samples. Resolution and efficiency are high in optimized methods, creating great potential for rapid detection and quantification [22]. Usually, the separation of analytes by CE is based on their different electrophoretic mobilities, which are strongly influenced by the composition of the background electrolyte (BGE), pH, ionic strength, concentration of organic cosolvents, and electrolyte additives [23]. The technique offers great possibilities for the analysis of real life samples.

CE has been widely used in the analysis of carboxylic acids. Methods have been developed to determine them in a diversity of matrices, including juice [24–26], wine [20,27,28], beer [29,30], coffee [31] and dairy products [12,22,32]. Few studies have dealt with carboxylic acids in fermentation samples of lignocellulosic material [10,15,33,34], and only one study has been made by capillary electrophoresis [35]. Soga and Ross [36] established a CE method for the analysis of 23 different organic acids from soy sauce, nutrient tonic and pineapple. For our analysis purposes this method was not efficient enough. The high pH would have caused problems because of very high concentration of sugars in the fermentation samples. Sugars are ionized at pH above 12 and can be seen in the electropherogram. In lower pH values the separation was not sufficient enough. Moreno et al. [20] introduced a CE method for the analysis of 9 organic acids from must, wine, brandy and vinegar. They used sodium tetraborate buffer and Ca^{2+} and Mg^{2+} as the metals to complex the carboxylic acids. Combination of these two alkali earth metals enhanced the separation significantly. Even so, using tetraborate buffer was not applicable in this study because most of the organic acids presented in this article could not be detected using this electrolyte solution. Combining these two CE methods gave the right separation efficiency.

The aim of this work was to develop a CE method for the simultaneous separation, identification and quantification of 18