Process Biochemistry 49 (2014) 1571–1579

Contents lists available at ScienceDirect

Process Biochemistry

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

Review

Aerobic submerged fermentation by acetic acid bacteria for vinegar production: Process and biotechnological aspects

Maria Gullo*, Elena Verzelloni, Matteo Canonico

Department of Life Sciences, University of Modena and Reggio Emilia, Via Amendola, 2, 42122 Reggio Emilia, Italy

ARTICLE INFO

Article history:
Received 4 March 2014
Received in revised form 19 June 2014
Accepted 4 July 2014
Available online 12 July 2014

Keywords:
Acetic acid bacteria
Gluconacetobacter
Acetobacter
Submerged fermentation
Acetic acid resistance

ABSTRACT

Strictly aerobic acetic acid bacteria (AAB) have a long history of use in fermentation processes, and the conversion of ethanol to acetic acid for the production of vinegar is the most well-known application.

At the industrial scale, vinegar is mainly produced by submerged fermentation, which refers to an aerobic process in which the ethanol in beverages such as spirits, wine or cider is oxidized to acetic acid by AAB. Submerged fermentation requires robust AAB strains that are able to oxidize ethanol under selective conditions to produce high-titer acetic acid. Currently submerged fermentation is conducted by unselected AAB cultures, which are derived from previous acetification stocks and maintained by repeated cultivation cycles.

In this work, submerged fermentation for vinegar production is discussed with regard to advances in process optimization and parameters (oxygen availability, acetic acid content and temperature) that influence AAB activity. Furthermore, the potential impact arising from the use of selected AAB is described.

Overcoming the acetification constraints is a main goal in order to facilitate innovation in submerged fermentation and to create new industry-challenging perspectives.

Contents

* Corresponding author. Tel.: +39 0 522 522063; fax: +39 0 522 522027.
E-mail addresses: maria.gullo@unimore.it (M. Gullo), elena.verzelloni@unimore.it (E. Verzelloni), matteocano91@live.it (M. Canonico).

http://dx.doi.org/10.1016/j.procbio.2014.07.003
1359-5113/

1. Introduction

Acetic acid bacteria (AAB) are strict aerobes that belong to *Alphaproteobacteria* and have the ability to partially oxidize carbon sources into a corresponding organic compound, such as ethanol to acetic acid [1,2]. This feature makes them valuable biocatalysts for a number of useful applications, but at the same time AAB are also spoiling organisms in some fermentation processes [3].

Acetic acid is the primary metabolite of AAB and is produced from the bioconversion of ethanol through two reactions catalyzed by the membrane-bound pyrroloquinoline quinone (PQQ)–dependent alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). ADH oxidizes the ethanol to acetaldehyde, which is then converted to acetic acid by ALDH and released into the surrounding environment. These two dehydrogenase complexes are strictly connected to the respiratory chain, which transfers electrons through ubiquinone (UQ) to oxygen, which acts as the final electron acceptor. The acetic acid produced by the partial oxidation of ethanol can be further oxidized in the cytoplasm by a set of soluble $NaD(P)^+$-dependent dehydrogenases (ADH and ALDH) via the tricarboxylic acid cycle, resulting in so-called acetate oxidation (overoxidation) [4].

Acetic acid is the main component in vinegar and is also recognized as an effective antimicrobial compound that prevents the growth of pathogenic and spoilage organisms in fermented foods; it also causes spoiling in beverages such as wine, in which it is detrimental even at concentrations as low as 1.2–1.4 g/L [5].

Food-grade vinegar, which is used worldwide as a preservative and condiment for food [6], is a diluted solution of acetic acid and is produced through a microbial oxidation carried out by AAB [7]. In addition, vinegar has been demonstrated to possess healthful properties [8].

Vinegar brewing can be performed by two main systems. The first system is solid-state fermentation (SSF), which uses microorganisms grown on substrates in the absence of free water; this system is used to produce vinegar from grains in Asian countries. SSF includes three main biological steps: starch liquefaction and saccharification, alcohol fermentation and acetic acid fermentation [9]. The second system is liquid fermentation, which comprises a set of techniques developed in Western and European countries. Among these techniques, the submerged system is used to produce vinegar at industrial scale [10] (Fig. 1).

A submerged system has several advantages over other techniques (e.g. SSF and surface fermentation), including high yield and process speed. Over the last few decades, many studies have examined process variables (oxygen availability, temperature, acetic acid and ethanol content), and a number of strategies for process control have been developed. As a result, acetic acid fermentation systems and the modern vinegar industry benefit from robust processes and optimization tools [11–15].

Major studies have also been conducted to examine the prevalent microflora, in order to determine the role of AAB in vinegar fermentation [16–24]. Differences in the species detected correlate with the selective pressure exerted by the acetic acid concentration of collection sites. In particular, highly acidic vinegar environments (acetic acid >6% (w/v)) favor the prevalence of *Gluconacetobacter* species, whose ADH shows a higher stability in high acetic acid content; in low acidity vinegars (acetic acid concentration ≤6% (w/v)) *Acetobacter* species are dominant, although *Gluconacetobacter* has also been found [25–27]. Although the aforementioned studies provided a good understanding of the ecophysiology of AAB in acidic niches, very little literature is available on the functionality of AAB in submerged processes relating to process parameters. The reasons for this lack of information can be mainly attributed to the difficulty of handling of AAB, resulting often in slow growing cultures, especially those derived from highly acidic vinegars.

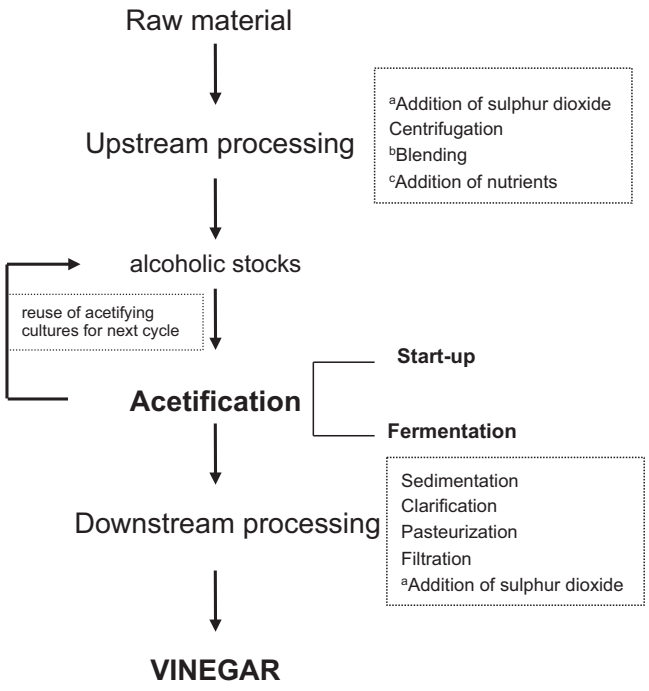

Fig. 1. Schematic representation of vinegar production in submerged system. [a] At concentrations specified by legislation; [b] blending with high acidity vinegar, to block undesired alcoholic fermentation; [c] nutrients containing carbon and nitrogen sources, vitamins and minerals are supplemented especially to produce high acidity vinegar (>12% of acetic acid) from alcoholic stocks containing no carbon sources except for ethanol [10].

In addition, it is well known that a large fraction of microorganisms present in both natural and industrial environments are uncultivable under standard laboratory conditions. Environments in which viable but not cultivable microorganisms have been found include soil [28], activated-sludge process for waste-water treatment [29], clinical samples exhibiting mixed communities of biofilm-forming bacteria [30], vinegars [31] and paper mill [32]. The uncultivability phenomenon limits the understanding of species richness and diversity of these environments and consequently a broad-spectrum strategy to select efficient strains as starter culture is affected.

The difficulty of cultivating AAB is one of the reasons why vinegar fermentation is still performed using unselected cultures.

Vinegar consumption has been increasing yearly worldwide [33], and understanding the microbial composition and activity of AAB in submerged conditions can result in further processes optimization, positively impacting production yield. Moreover, consumer demand for high added-value products, including fermented and low sour beverages indicates potential applications for novel and functional starter cultures.

The present review aims to outline the main features of the aerobic submerged process for vinegar production at the industrial scale and to overcome acetification constraints in order to further enhance processes optimization.

2. Aerobic submerged fermentation

AAB are exploited for the commercial production of a variety of biomolecules including dihydroxyacetone [34], 2-keto-L-gulonic acid, D-sorbitol [35], gluconic acid [36], using submerged fermentation (SF) processes.

SF for vinegar production is an aerobic process by which the ethanol in liquids such as spirits, wine or cider is oxidized to acetic acid by AAB, in controlled stirring conditions [37].