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Bioconversion of spent coffee grounds into carotenoids and other valuable metabolites by selected red yeast strains

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ABSTRACT

Spent coffee grounds (SCG) represent the main coffee industry residues with a great potential to be reutilized in various biotechnological processes. In this study, several carotenogenic yeasts strains were exploited for the production of vitamin-enriched biomass, cultivating in SCG-based media. The fermentation was firstly carried out in Erlenmeyer flasks in order to select the best biomass and pigment producer. Among four tested strains, *Sporobolomyces roseus* showed the highest potential for the accumulation of carotenoids. Maximum pigment concentration and yield was obtained when cultivating in SCG-based media, 12.59 mg l^{-1} and 1.26 mg g^{-1}, respectively. Comparing both, the batch and the fed-batch cultivation modes, the strategy of sequential addition of pre-concentrated SCG media in the bioreactor gave higher biomass yield (maximum 41 g l^{-1} during 41–48 h after the beginning of fermentation). Thus, SCG can be considered as potentially promising industrial waste stream for economically feasible production of enriched yeasts biomass.

1. Introduction

Carotenoids are tetraterpenoids, consisting of highly unsaturated isoprene derivatives. Generally, those compounds are the most widely distributed class of pigments in nature, displaying yellow, orange or red color [1]. They act as membrane-protective antioxidants which efficiently scavenge $^1O_2$ (singlet molecular oxygen) and peroxyl radicals and the antioxidant efficiency is apparently related to their specific structure. Carotenoid pigments occur universally in photosynthetic systems of higher plants, algae and phototrophic bacteria. In non-photosynthetic organisms, carotenoids are important in protecting against photo-oxidative damage. Thus, many non-phototrophic microorganisms rely on carotenoids for the protection when growing in the light and in the presence of air [2].

Carotenoids are produced primarily by filamentous fungi and yeasts but also by some strains of bacteria, algae and lichens. Carotenogenic (red) yeasts are diverse assemblage of unrelated organisms capable of carotenoids biosynthesis. Most of them belong to the *Basidiomycota* phylum growing on the phyllophane or on the decaying plant material. Many red yeast species, which belongs to the *Rhodotorula*, *Rhodosporidium*, *Sporidiobolus*, *Cystofilobasidium* and *Phaffia* genus, are considered as potential candidates for the biotechnological production of carotenoids [3].

Pigments are commercially used as food colorants, animal feed supplements and, more recently, as nutraceuticals for cosmetic and pharmaceutical purposes. The market demand for carotenoids is anticipated to increase substantially, since carotenoids exhibit significant anti-carcinogenic activities and play an important role in the prevention from chronic diseases [4]. The biotechnological production of carotenoids can become industrially feasible if the cost of the process is minimized by the utilization of cheap carbon substrates such as waste products of agriculture or food industry. Moreover, the utilization of these waste substrates for the carotenoids production might reduce the environmental and energetic problems related to their disposal [5].

After petroleum, coffee is the second largest traded commodity in the world and; therefore, the coffee industry is responsible for the generation of large amount of waste residues [6]. Spent coffee ground (SCG) are the solids obtained by the treatment of raw coffee with hot water or steam to prepare instant coffee, and SCG worldwide generation is estimated to reach 6 million tons per year [7]. Despite the fact that SCG has been (due to its high calorific power), used in industrial boilers, the most of this residue remains unutilized, being discharged to the environment or burned, which cannot be considered either environmentally friendly or

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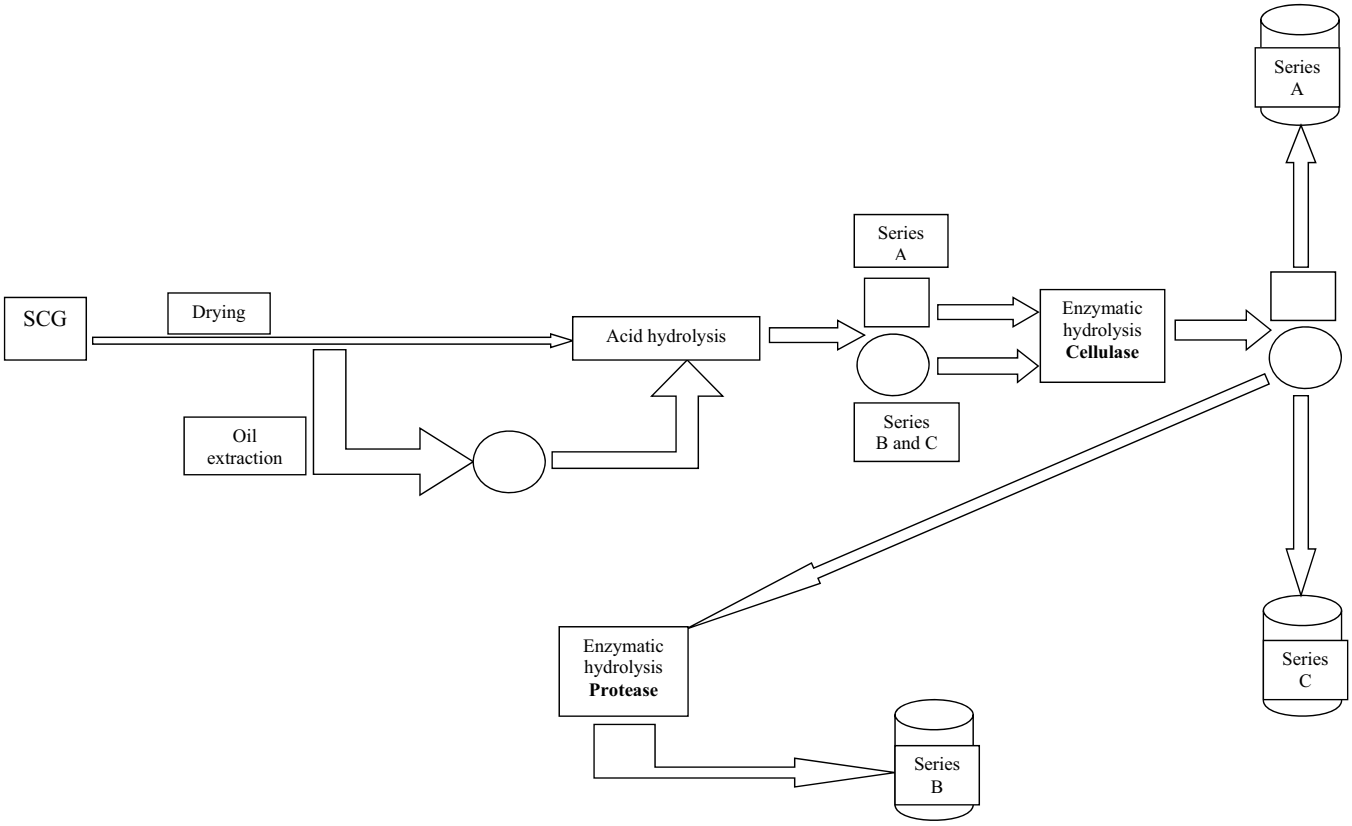

Fig. 1. A schematic preview of the preparation of various SCG-based media series. Series (A) SCG-based media prepared without oil extraction, just acid hydrolysis, treated with cellulase enzymes; Series (B)SCG-based media prepared with oil extraction, acid hydrolysis and treatment with cellulases and proteases; Series (C)SCG-based media prepared with oil extraction, acid hydrolysis and treatment with cellulase enzymes.

economically feasible technique [8]. Therefore, the utilization of SCG as a raw material for the production of ethanol [9,10] biodiesel [11,12], polyhydroxyalkanoates [13] or polyphenolic compounds [8] are recently investigated.

The objective of this work was to investigate the ability of selected red yeasts strains, such as *Sporobolomyces roseus*, *Rhodotorula mucilaginosa*, *Rhodotorula glutinis* and *Cystofilobasidium capitatum*, to utilize SCG as the only carbon source and, thus, to accumulate high value metabolites—carotenoids and ergosterol. Screening experiments, focused on the strain selection and the growth media composition optimization, were performed in Erlenmeyer flasks, while further experiments were carried out in laboratory bioreactor.

2. Methods and materials

2.1. Raw material preparation

Spent coffee ground (SCG) was obtained from a coffee automat machine at the Faculty of Chemistry, Brno University of Technology, Czech Republic. The waste material was firstly dried to the constant weight (80 °C for 24 h) resulting in the moisture content less than 5%. A part of dried SCG was directly utilized for the cultivation experiments (Fig. 1) while the rest of the material was further used for oil extraction with *n*-hexane in Soxhlet extractor apparatus as described by Al-Hamamre et al. [11]. SCG (either after or without oil extraction) were then pretreated with acid and thereafter subjected to the enzymatic hydrolysis. To hydrolyze hemicelluloses of the raw material, SCG were firstly treated by 1% H_2SO_4 (vol/vol) for 90 min at 121 °C [9]. In the next step, prior to the red yeasts cultivation, the SCG material was subjected to the enzymatic decomposition. The enzymatic digestion was used for breaking up the complex SCG matrices and to hydrolyze the crystalline cellulose structure releasing fermentable monosaccharides and other compounds important for the yeasts growth. Therefore, pH of the suspension after the acid hydrolysis was set to 4.5 by 10 M NaOH and cellulose was executed by 0.5% (vol/vol) of Celluclast 1.5 L (Novozymes A/S, Bagsværd, Denmark) at 50 °C under permanent shaking (150 rpm) for 24 h [12]. Afterwards, the pH was set to 7.0 by 10 M NaOH and one part of the suspension was treated with commercially available protease Alcalase (activity $U = 6.22\ \mathrm{U\,l^{-1}}$, Alcalase, Clea, The Netherland) for 24 h at 50 °C under permanent shaking. At the end of the process, the solids, obtained after different enzyme treatments, were removed by filtration and the permeates were utilized for SCG-based media preparation and further red yeasts cultivations. The diagram of the SCG treatment is provided in Fig. 1.

2.2. SCG-based media composition analysis

Various analytical techniques and methods were used in this study in order to determine the composition of SCG-based media prior to the yeast cultivation. The liquid phase obtained from 15% (w/v) of enzymatically hydrolyzed and oil extracted SCG was further analyzed for several parameters, such as: total and individual sugar content, total phenolics, nitrogen and phosphorus content, conductivity, ash and dry matter content.

The concentration of dry matter was estimated by drying 10 ml of SCG-based media to constant weight at 105 °C. Ash content was determined as the weight of solids obtained after incubation of 2 ml of SCG-media at 800 °C for 2 h. Subsequently, the phosphorus content in ash was measured by Ion Chromatography (850 Professional IC, Metrohm, Switzerland) using Metrosep