Second Generation Bioethanol Production from Household Food Wastes via a Newly Isolated Yeast Strain of *Wickerhamomyces Anomalus*

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Abstract
In the current study the efficiency of second generation bioethanol production from the pre-dried and shredded organic fraction of household food waste was investigated using the newly isolated yeast *Wickerhamomyces anomalus* X19. Separatete hydrolysis and fermentation (SHF) as well as simultaneous saccharification and fermentation (SSF) experiments were conducted at batch mode. Different loadings of cellulolytic enzymes as well as different mixtures of cellulolytic with amylolytic enzymatic blends were tested in order to enhance the substrate saccharification and conversion efficiency, leading to promising ethanol yields and productivities.
Keywords: bioethanol, food wastes, *W. anomalus*

1. Introduction

Biomass - based fuels, the so-called biofuels, are regarded among the key renewable energy sources that can contribute to the sustainable development of economies. Among them bioethanol and its blends has been most widely used as alternative liquid fuel for transportation. Sustainable ethanol production however can only be assured if it is based on the exploitation of biomass types that are abundant, renewable and low-, or even, zero-cost. Food wastes (FW) are zero cost, rich in carbohydrates and are generated in huge quantities annually in both developed and developing countries. Moreover FW if not treated properly end up in landfills, were they decompose slowly releasing greenhouses gasses and affecting ecological balances (FAO, 2013).

In the present study a FW generated at consumer level i.e. the organic fraction of household food waste (food residue biomass, FORBI) was assessed as feedstock for the production of bioethanol. As biocatalyst a novel yeast strain, *Wickerhamomyces anomalus* X19, a physiologically versatile yeast strain, capable of growing under low pH, high osmotic pressure and anaerobic conditions, was used.

2. Materials and Methods

2.1. Feedstock

FORBI was collected at municipality from 240 houses of the Municipality of Chalandri, Athens, Greece. Upon collection FORBI was heat dryed at 80^{o}C and milled, resulting to a homogeneous product with high carbohydrate content (soluble sugars, 0.21±0.02 g/g TS, total carbohydrates, 0.43±0.03 g/g TS, complete characterization in Ntaikou et al., 2018).

2.2. Enzymatic saccharification

Enzymatic treatment was performed in duplicates with FORBI at pH 4.8, 50 °C and solid loading 10 % wTS/v using either a commercial cellulase blend (CE) (Cellic CTec2-CEL, Sigma-Aldrich) at 2-30 FPU/g TS, or 5 enzymatic mixtures of CE, commercial fungal α-amylase (C.A.) and amyloglucosidase (A) (Table 1).

Table 1. Composition and enzymatic loadings of enzymatic mixtures used for the hydrolysis of FORBI

Mixture	CE FPU/g TS	CA U/g TS	A U/g TS
1	0	10	5
2	30	2	0
3	30	2	5
4	30	10	0
5	30	10	5

2.2. Fermentation tests

In the present study, the yeast strain *W. anomalus* X19 (MH237950.1) that was isolated recently from decaying wood was used (Imen Ben Atitallah et al, 2019a). FORBI was used as the sole carbon source at solids loading 10% (wTS/v). All fermentation tests were carried out duplicate in batch mode under sterile and anaerobic conditions, at 30 °C and 150 rpm. Fermentation media were supplemented with KH_2PO_4, $MgCl_2.6H_2O$, $(NH_4)_2SO_4$ 1 gL^{-1} each (Ntaikou et al., 2018). For simultaneous saccharification and fermentation experiments (SSF) cultures were also supplemented with the enzymatic mixtures 3 and 5. For separate hydrolysis and fermentation experiments (SHF), the same enzymatic mixtures were added to the

medium and the hydrolysis step was performed at 50°C for 28h. The enzymes were then inactivated prior to inoculation.

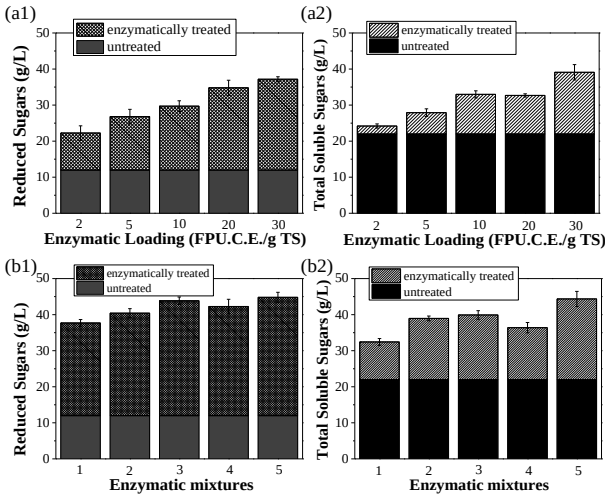
2.4. Analytical methods

The following parameters were quantified as described in the study of Ntaikou et al (2018): total solids (TS), volatile solids (VS), Total Kjeldahl Nitrogen (TKN), crude protein, total and soluble carbohydrates, starch, cellulose, glucose and ethanol. Reducing sugars were also quantified as described by Ben Atitallah et al. (2018).

3. Results

3.1. Enzymatic hydrolysis of FORBI

The effect of enzymatic loading and types of enzymes used in enzymatic mixtures during hydrolysis of FORBI is presented in Fig.1. Total soluble (free) sugars as well as the reducing sugars that were released after 48h of hydrolysis (for CE) and 30h of hydrolysis (mixtures) are presented. Based on the solid loadind used in the experiments (10%) and composition of FORBI, the maximum concentration of soluble sugars that could be achieved is ~35g/L and~ 45g/L if holocelluse or both hollocellulose and starch are hydrolysed, respectively. It is apparent that CE leads to partial saccharification since it does not affect starch. However it seems that the highest enzymatic loading of CE leads to the maximum theoretical sugars release, whereas with almost all them being reducing (Fig.a1 and a.2). When enzymatic mixtures were used the maximum theoretically estimated liberation of sugars was achieved for mixture5 (maximum loadings of CE, CA and A).



3.2. Ethanol production from FORBI via W. anomalus

The production of ethnol from FORBI was assessed using the enzymatic mixtures 3 and 5 (cellulolytic and amylolytic) at SSF and SHF mode. The results are presented in Table 2, making apparent that the fermentation mode do not seem to affect the final yields. A small increase of ~4% is observed when higher enzymatic loading of CA was used for the hydrolysis in both SSF and SHF mode. Ethanol yields are higher than those achieved in the study of Ntaikou et al (2018) in which *S. saccharomuces* and *P. stipites* were used for ethanol production from FORBI, indicating that *W. anomalus* is more efficient ethanol producer. Ben Atittalah et al (2019b) during assessing ethanol production from potato peels (PP) via *W. anomalus* had shown that yields could be enhanced with SHF process. It should be noted however that PP is a plant based raw material, whereas FORBI is a mixture of raw and processed food wastes that might be more vulnerable to direct hydrolysis even at mild conditions, as those imposed during SSF processes.

Table 1. Ethanol yields from the fermentation of FORBI at SSF and SHF schemes using *W. anomalus*

Enz. Mixture	process	YEtOH (g EtOH/g FORBI)	YEtOH (g EtOH/g total sugars)
3	SSF	0.147±0.001	0.342±0.001
	SHF	0.146±0.001	0.340±0.001
5	SSF	0.153±0.001	0.355±0.001
	SHF	0.151±0.002	0.351±0.002

4. Conclusions

The complete hydrolysis of the complex carbohydrates contained in FORBI can be achieved using enzymatic mixtures of cellulolytic and amylolytic enzymes. Fermentation of FORBI via *W. anomalus* leads to similar yields whether it is performed in SSF of SHF mode. In all cases high ethanol yields were achieved, indicating that FORBI is a promising substrate for ethanol production.

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