



# Optimization of thermo-chemical pretreatment and enzymatic hydrolysis of kitchen wastes



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## ABSTRACT

The use of abundant waste materials with high carbohydrate content may contribute substantially to reduction of biofuels production cost. The present study aimed at optimizing the combined effect of thermo-chemical pretreatment and enzymatic hydrolysis of kitchen wastes (KW) for maximizing the production of fermentable soluble sugars. To this end, acid pretreatment of KW samples was performed with hydrochloric acid (0–3% HCl) at 30–100 °C for 0–120 min treatment time. Alternatively, alkaline pretreatment of KW samples was performed with potassium hydroxide solution (0–11%) at constant temperature and time (0 °C and 20 min, respectively). KOH pretreatment at such conditions targets to degrade the resistant starch of KW samples. Both acid and alkaline pretreatments were followed by addition of variable levels of enzyme dosage (0–3.6% v/v  $\alpha$ -amylase and 0–3.2% v/v amyloglucosidase-AMG) at constant pH, temperature and time (pH = 5,  $T = 50$  °C and  $t = 30$  min, respectively). Based on our results, glucose concentration increased by ~300% after pretreatment with either acid or KOH in combination with enzymatic hydrolysis (2% HCl, 85 °C, 80 min, 0.1%  $\alpha$ -amylase, AMG, and 1% KOH, 0 °C, 20 min, 1.1%  $\alpha$ -amylase, 0.4% AMG) compared to raw (untreated) KW. Estimating the different  $Y_G$  yields at KW loading of 5%, an increase of 192% and 121% for total soluble monosugars and total soluble sugars, respectively, was succeeded compared to untreated KW. The effect of solids loading on the obtained sugar yields using the optimum conditions for thermo-chemical pretreatment followed by enzymatic hydrolysis was also tested resulting to 27.5% increase of the soluble glucose yield when half of the solids loading (2.5%) was used. A decrease of total soluble sugars yield by 32.2% was observed when solely acid hydrolysis at optimum conditions from our previous study was applied at 30% solids loading.

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## 1. Introduction

Growth in the EU is still accompanied by increasing amounts of waste, causing environmental damage, negative effects on health and quality of life and unnecessary losses of materials and energy. It is a strategic goal of the EU to reduce these negative impacts, turning the EU into a resource efficient “Recycling Society” according to the “Green Paper on the management of bio-waste in the European Union” (2008). Food and kitchen waste (KW) from households, restaurants, caterers and retail premises, and similar waste from food processing plants are considered major contributors to bio-wastes. In the most recent study from European Commission DG ENV (2011) the total bio-waste generation between 2013 and 2020 at the EU27 was projected to be 741.607 ktonnes (collected) with the total annual increase estimated at 88.7–96.3 Mt. In Greece, an average amount of 456 kg Municipal Solid Wastes (MSW) per capita per year are generated (Eurostat, 2011), consisting mainly

of fermentable organic material (47%) and paper residuals (20%) (Bosdogianni, 2007).

Waste management options for kitchen wastes, as part of bio-wastes, include, in addition to prevention at source, collection (separately or with mixed waste), anaerobic digestion (AD) and composting, incineration, and landfilling. Composting is the preferred waste treatment method, at an average EU27 level, receiving approximately 31% of the projected 741.607 ktonnes (European Commission DG ENV, 2011), Mechanical Biological Treatment (MBT) 20% and landfilling 19.2%. However, a concern has been expressed by some stakeholders that a kitchen waste recycling target focusing on composting and AD would ignore the potential benefits of other innovative ways of gaining energy from kitchen waste, such as biorefining. The IEA Bioenergy Task 42 on Biorefineries (2013) has defined biorefining as the sustainable processing of biomass, KW in our case, into a spectrum of bio-based products (food, feed, chemicals, materials) and bioenergy (biofuels, power and/or heat). It is expected that this market segment will grow in the future and that this will affect the composition, the amounts and the use of different biomass waste streams especially because it is also supported by the existing European legislative

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framework. The Directive 2003/30/EC of the European Parliament and of the Council (2003), which aims at the progressive use of bio-fuels or other renewable fuels for transport, leads to international, national and regional level to focus on alternative energy sources. To this end, the European Commission has proposed indicative targets for 10% biofuels in transportation fuels by 2020 (European Commission, 2008). Moreover, the European Commission Landfill Directive (1999) by strongly promoting the minimum use of landfills in European countries has succeeded to reduce the share of landfilling in the EU-27 from 68% in 1995 to 38% in 2008, with the landfill rate in Greece being 81% in 2008 (Eurostat, 2011). In any case, the environmental and economic benefits of different bio-waste treatment methods depend significantly on local conditions such as population density, infrastructure and climate as well as on markets for associated products (energy and composts).

Recent studies focus on the increasing exploitation of carbohydrate-rich organic wastes, such as kitchen waste, which can relatively easily be converted using various biological processes into valuable products like ethanol (Wang et al., 2008; Moon et al., 2009), lactic acid (Sakai and Ezaki, 2006), succinic acid (Praneetatananon et al., 2005) and hydrogen (Han and Shin, 2004). Among other bio-based compounds bioethanol, in particular, produced from renewable sources and especially from lignocellulosic biomass (*second-generation biofuel*) is considered as a solution to the worldwide energy problem by supplying fuel sustainably, affordably, and with great environmental benefits (decreasing urban air pollution, reducing accumulation of carbon dioxide, etc.). In fact, production of fuel bioethanol constitutes 60% of world's total ethanol production (Thomsen et al., 2003), while other users include beverage industry and other industrial practices.

The KW fraction of MSW consisting mainly of carbohydrates, either in soluble or polymeric form (starch, cellulose, hemicellulose) and other organics (proteins, lipids, acids, etc.), represents an alternative and valuable source of amenable to fermentation sugars and then into valuable products, such as gaseous and liquid biofuels (Li et al., 2007; Ruggeri and Tommasi, 2012). However, hydrolysis of solids in KW is still recognized as a rate-limiting step in their application for biological processes. Numerous methods (e.g. thermal, microwave and ultrawave pretreatment) have been proposed to enhance solubilization of the complex particulate organic substrate of KW accelerating the hydrolysis rate (Sawayama et al., 1997; Marin et al., 2011). Hydrolysis of starch components, as those contained in KW, may result into the breakage of glycoside bonds of polysaccharides emerging oligosaccharides ( $3 < n < 10$ ) and/or maltodextrins ( $2 < n < 20$ ) and monosaccharides, which can be further fermented into valuable bio-products. Chemical hydrolysis of starch, solely (Vavouraki et al., 2013) or under the action of ultrasonic field or strong mechanical stresses (Losev et al., 2003) may result into degradation of starch glycosidic bonds. When the environmental vector was taken into account along with the technical and the economic evaluation towards the sustainable decision making process for the most suitable pretreatment method, the mechanical (e.g., pressurization–depressurization) and chemical methods were proved to be the most appropriate alternatives prior to anaerobic digestion (Carballa et al., 2011). Enzymatic hydrolysis of KW prior to fermentation has seldom been applied as a pretreatment method for their solubilization and hydrolysis due to the excessive cost of enzymes which may render the overall process economically unsustainable. However, several experimental studies of enzymatic hydrolysis have focused on the biodegradability improvement of residential and industrial food wastes by optimizing the conditions of simultaneous saccharification and fermentation (SSF) for ethanol production (Kim et al., 2006; Moon et al., 2009; Uncu et al., 2011; Yan et al., 2012). Kim et al. (2006) showed that a mixed enzyme treatment of food waste exhibited better reduction efficiency of the volatile suspended solids (VSS)

than a single enzyme treatment, with the highest VSS reduction observed at an enzyme mixture ratio of 1:2:1 (carbohydrase:protease:lipase, respectively). A mixture of commercial available enzymes of amyloglucosidase and carbohydrase was used by Moon et al. (2009) for enzymatic hydrolysis of food waste broth prior to fermentation by *Saccharomyces cerevisiae* strain KCTC 7107 for ethanol production. The enzymatic saccharification of food waste in batch mode improved the reducing sugars concentration in the hydrolysates and eventually the ethanol fermentation by *S. cerevisiae* in the work of Yan et al. (2011). Yan et al. (2012) extended their work to a fed-batch hydrolysis process using a mixture of commercial available enzymes (amyloglucosidase and  $\alpha$ -amylase) followed by fermentation with *S. cerevisiae* H058. Pretreatment of the polymeric carbohydrate material is expected to improve the rate of subsequent enzymatic hydrolysis and increase the yields of bio-products. Towards this direction only very limited studies have investigated the effect of chemical pretreatment to mixed enzymatic hydrolysis for fermentation-type experiments of food wastes, e.g. Cekmecelioglu and Uncu (2012). The authors however concluded that a pretreatment method prior to enzymatic hydrolysis is not strictly needed for production of high glucose levels from the kitchen wastes tested in their study.

To the best of our knowledge there is no systematic study on the optimization of chemical pretreatment prior to enzymatic hydrolysis of kitchen waste. Thus, the aim of this work was to evaluate specific combined schemes of chemical with enzymatic treatment in order to optimize the hydrolysis of a representative sample of KW by maximizing the production yield of fermentable soluble sugars and then compare the optimum results with the best ones concluded in our previous study (Vavouraki et al., 2013), where exclusively chemical treatment was used for the hydrolysis of the same KW sample. More specifically, our goal was to use for the experimental design and optimization a genetic algorithm (GA) for multi-objective experimental optimization followed by cost estimation of the tested experimental parameters combination in order to estimate the optimum values of the most critical parameters (acid or base concentration, temperature, residence time and enzymes concentration) in our hydrolytic experiments in the attempt to boost degradation of such a carbohydrate-rich material, such as KW, to fermentable soluble sugars.

## 2. Materials and methods

### 2.1. Kitchen wastes (KW)

The kitchen wastes (KW) used in the present study were collected from the Students' restaurant at the Students Centre of the National Youth Foundation, which is located at the University of Patras (Greece). The KW composition was 50% raw-fresh food (46.8% vegetables, 3.2% minced meat), 15.6% cooked food (4.2% meat, 0.9% fish, 2.1% legumes, 3.7% potatoes, 1.9% rice and 2.8% others), 14.5% fruits, 9.7% salads, 8% bread and 2.2% dessert (on a wet-weight basis). Mixed KW was homogenized using laboratory cutting equipment (Sorvall) and then stored at  $-20^{\circ}\text{C}$ , during the whole experimentation period, in order to maintain its physico-chemical characteristics. Prior to KW pretreatment a homogenized wet sample (30–50 g) was dried at  $55^{\circ}\text{C}$  for 24 h or more, until constant weight, and then was ground with a ceramic mortar and pestle and sieved (final particle size  $<1\text{ mm}$ ). More details can be found in our previous study (Vavouraki et al., 2013).

### 2.2. Chemical and enzymatic analysis

A representative sample of KW was characterized for moisture, residual ash, total and volatile solids, oil and grease, total nitrogen

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