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Use of *Carica papaya* enzymes for enhancement of H_2 production and degradation of glucose, protein, and lipids

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Abstract

Anaerobic batch experiments were carried out to examine the effect of supplementation of mixed culture bacteria with *Carica papaya*as enzymes for enhancement of hydrogen yield from degradation of glucose, protein, and lipids. The results showed that hydrogen yield (HY) based on protein and lipids degradation increased from 52.2 ± 7.5 to 130.6 ± 8.5 ml/$g_{protein}$, and from 43.0 ± 5.3 to 64.8 ± 3.1 ml/g_{lipid} respectively with addition of *Carica papaya* as enzymes source. This corresponded to substrate degradation efficiency of 51.3 ± 4.4% for protein and 33.7 ± 2.6% for lipids. However, the hydrogen yield and degradation efficiency of glucose was slightly improved by addition of *Carica papaya*as enzymes.

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Keywords: *Carica papaya; hydrogen yield; metabolite products*.

1. Introduction

One of the well-known renewable resources is bio-hydrogen that can be easily used in fuel cells for electricity generation [1]. In this concern, anaerobic digestion (AD) is the most promising technology for hydrogen production from biodegradable wastes. However, hydrolysis process is the rate-limiting step during AD [2]. Lipids and proteins have low anaerobic biodegradability [3]. Whereas, protein and lipids degradation leads to the accumulation of ammonia and long chain fatty acids (LCFAs), respectively which are important inhibitors of the anaerobic consortium microorganisms [4]

Enzymes supplementation was earlier exhibited a great positive impact on organic degradation process [5]. It has also been used to increase the hydrolysis during or prior to anaerobic digestion process [6]. Mixed bacterial cultures comprising Bacillus sp. as a protease and amylase producer and Pseudomonas aeruginosa and Acinetobacter calcoaceticus as lipase producers were used in the treatment of lipid-rich wastewater. Results showed reduction in biological oxygen demand (BOD) value and lipid content reached more than 99% within 12 days under aerobic conditions [7]. Anaerobic digestion of pot ale residues containing intact yeast cells was pre-treated with lytic enzymes and showed COD reductions of

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87%, compared with only 13% without enzymes [8]. These findings imply the capability of different types of enzymes on degradation processes in anaerobic ecosystems. However, the strategy of enzymes enrichment has some drawbacks such as its high costs, thus it was not economically feasible [5].
Therefore, nature source of degrading enzymes is preferable and promising approach. *Carica papaya plants*, grows in a wide range of climate, makes it a cheaper natural enzyme source and has the potential additive in detergents [9]. The latex of *Carica papaya* is well known for being a rich source of the four cysteine endopeptidases papain, chymopapain, glycylendopeptidase and caricain [10]. As well as, it was found to be plant source of lipases [11]. Chaiwut et al. obtained a substantial extraction of proteases from papaya peels [12]. Therefore, the main objectives of this study is to examine the effect of addition *Carica papaya* latex and peels as an enzymes source on the hydrogen yield and degradation efficiency of glucose, protein, and lipids.

2. Methodology

2.1. Feedstock preparation and seed microflora:

The substrate was prepared by using D-Glucose anhydrous (Fisher Co.), bovine serum albumin (Sigma-Aldrich Co.). The edible oil was the lipids source. *Carica papaya* latex and peels slurry was prepared at a ratio of 1:2 (v/v) between latex and peels. The aforementioned mixture was crushed and homogenized using an electrical blender. The characteristics of papaya latex and peels mixture was pH = 4.03; COD_{total} = 28.1 g/l; TS = 30.7%; VS = 28.8%; TKN = 1.1g/l; C/N ratio = 25.5; carbohydrate = 14.7g/l; and lipid = 6.2g/l.
Seed microflora obtained from the thickener tank of a wastewater treatment plant situated in Alexandria city, Egypt. The volatile suspended solids (VSS) was 19.28 g/l. The mixed culture bacteria were pre-heated at 100 °C for 15 minutes to inhibit the bioactivity of hydrogen consumers [13].

2.2. Batch experimental setup

Batch anaerobic experiments were conducted in triplicate using a series of 200 ml serum bottles with working volume of 150 ml as depicted in Table 1. Initial substrate to inoculum ratio designed to be in range of 6 to 8 g_{COD}/g_{VSS} in all batches. Whereas, maximum hydrogen production was achieved in this range according to previous studies [14]. The COD conversion factors, used in this study, were 1.07 $g_{COD}/g_{carbohydrate}$, 1.50 $g_{COD}/g_{protein}$, and 2.91 g_{COD}/g_{lipid}. All batches were initially flushed with nitrogen for 2 minutes to remove the oxygen from the culture medium, headspace and capped tightly with rubber stoppers and aluminium caps. The pH of the feedstock was adjusted to 7.0 ± 0.2 using sodium bicarbonate. The reactors were incubated under thermophilic condition (55 ± 2 °C).

Table 1. The experimental set-up

Batch no.	Experiments set-up
G1	4.2 g Glucosein 150 ml Deionized water
G2	4.2 g Glucosein 120 ml Deionized water + 30 ml Inocula
G3	4.2 g Glucosein 110 ml Deionized water + 30 ml Inocula + 10 ml Papaya
P1	3.0 g Protein in 150 ml Deionized water
P2	3.0 g Protein in 120 ml Deionized water + 30 ml Inocula
P3	3.0 g Protein in 110 ml Deionized water + 30 ml Inocula + 10 ml Papaya
L1	1.5 g Lipid in 150 ml Deionized water
L2	1.5 g Lipid in 120 ml Deionized water + 30ml Inocula
L3	1.5 g Lipid in 110 ml Deionized water + 30ml Inocula + 10 ml Papaya

2.3. Analytical methods

Total solids (TS), volatile solids (VS), chemical oxygen demand (COD), Total Kjeldahl nitrogen (TKj-N), and lipids were quantified according to APHA [15]. Protein was calculated as follows (6.26 × TKj-N) [16]. Glucose was measured according to the phenol–sulfuric acid method [17]. Volatile fatty acids (VFAs) concentrations in the liquid samples were analysed by high performance liquid chromatography (LC-10AD, Shimadzu, Japan). The temperature of column oven was 40 °C. 4 mM H_2SO_4 was used as a mobile phase at a flow rate of 0.5 ml/min for 22 min followed by 0.4 ml/min for 8 min.
The evolved gas was measured by displacement method. Moreover, Hydrogen content in the biogas was analysed using a gas chromatograph (GC-2014, Shimadzu, Japan) equipped with a thermal conductivity detector and Shin carbon column. The operational temperatures of the injection port, the column oven and the detector were 100, 120 and 150°C, respectively. Helium was used as the carrier gas at a flow rate of 25 ml/min.

3. Results and discussion

3.1. Hydrogen productivity

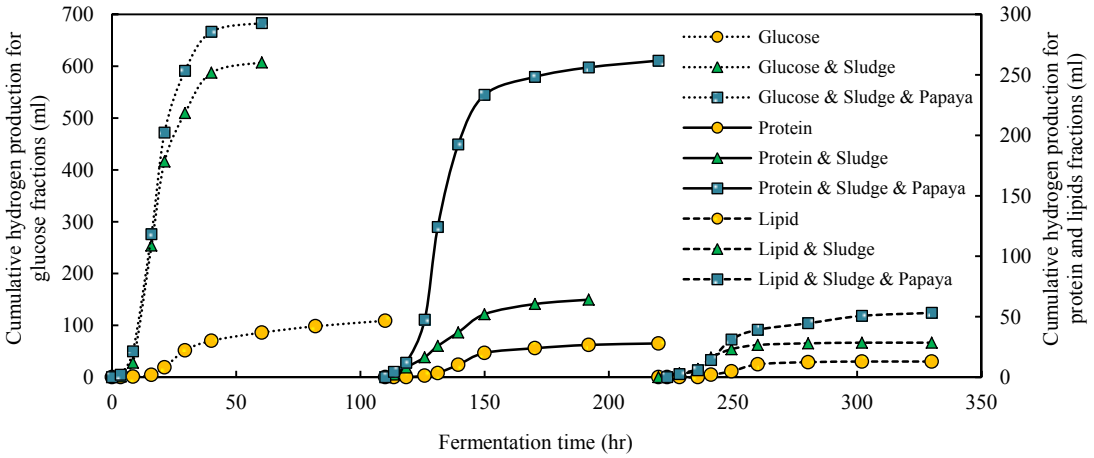

Fig. 1.Effect of *C. papaya* on cumulative hydrogen production from glucose, protein, and lipid fractions.

Fig. 1 shows the cumulative hydrogen production (CHP) from glucose, protein and lipids using mixed culture bacteria. The results obtained indicated that the CHP was the highest for glucose (610.8 ± 34.8 ml) as compared to 64.1 ± 11.9 ml for protein and 23.5 ± 2.9 ml for lipids. This indicates that the hydrolysis of protein and lipids are the rate limiting step. High hydrogen production abundantly developed from AD of glucose as the carbon source referred to its easily biodegradable. However, the reasons behind hardly bio-H_2 produced from proteins and lipids owing to: 1. Protein in its native folded conformation is insusceptible to protease cleavage [18]; and 2. β-oxidation (the degradation of long-chain fatty acids, the main constituent of lipids) cannot generate hydrogen [19].
However, the addition of *Carica papaya* enzymes improved the CHP by a value of 261.8 ± 27.2 ml for protein and 42.8 ± 3.7 ml for lipids as shown in Fig. 1. On the other hand, The CHP was remained unaffected by the addition of *Carica papaya* enzymes to glucose. The tremendously CHP increase in protein batches is mainly due to adding of *C. papaya* which contains many types of proteolysis enzymes such as proteases and papain. Silva et al. [20] proved that during *C. papaya* latex coagulation with

protein, several peptides are processed in an orderly fashion. In addition, papain is characterized by its ability to hydrolyse large proteins into smaller peptides and amino acids. Similarly, CHP from lipids fraction was extremely augmented due to supplemented by *C. papaya*. This was a result of lipases existence in its latex. Lipases are enzyme sets that catalyse the hydrolysis of lipids [11].

3.2. Substrate conversion and hydrogen yield

Glucose, proteins, and lipids removal efficiencies increased from 37.2 ± 1.0 to 58.3 ± 3.5, from 30.2 ± 2.7 to 32.8 ± 1.6, and from 23.3 ± 1.3 to 29.7 ± 1.6% respectively by providing seeding sludge to each individual batch media as shown in Fig. 2. However, this removal reached up to 60.9 ± 4.0, 51.3 ± 4.4, and 33.7 ± 2.6% respectively with *C. papaya* addition as enzymes source. The high increase in protein degradation is owing to the presence of papain and protease enzymes in *C. papaya*. Whereas, catalytic activity of papain involves hydrolysis of proteins with broad specificity for peptide bonds [21]. Moreover, lipase from *C. papaya* Latex has been regarded as a "naturally immobilized" biocatalyst [11].

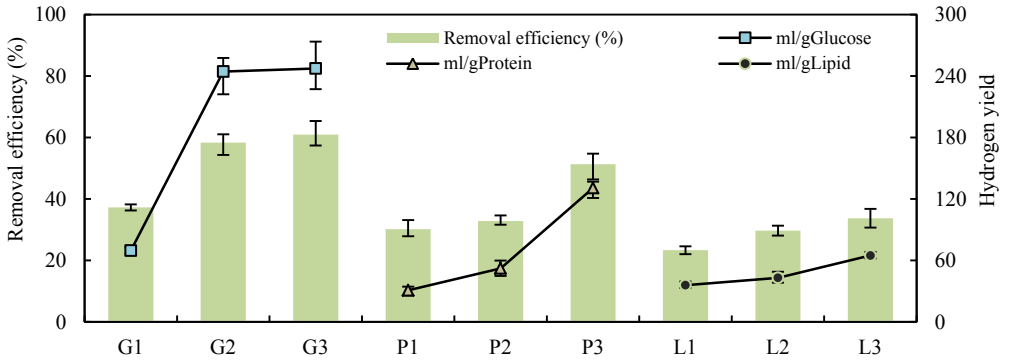

Fig. 2.Hydrogen yields and removal efficiencies of different fractions.

As depicted from Fig. 2, HY based on glucose, protein and lipid conversion were considerably increased from 69.5 ± 3.7 to 244.4 ± 19.4 ml/$g_{glucose}$ (0.56 to 1.97 mol/$mol_{glucose}$), as well as a bit increased from 30.8 ± 4.5 to 52.2 ± 7.5 ml/$g_{protein}$, and from 35.8 ± 3.3 to 43.0 ± 5.3 ml/g_{lipid}, when each fraction supplemented by mixed inocula, respectively. Further addition of *C. papaya* leads to a slight increase in HY i.e. 247.3 ± 23.8 ml/$g_{glucose}$(1.99 mol/$mol_{glucose}$) in glucose batches. On contrary, a high increase in HY based on protein and lipid conversion as they became 130.6 ± 8.5 ml/$g_{protein}$ and 64.8± 3.1 ml/g_{lipid}, respectively. Similar finding was observed by Fang et al. [22] who recorded HY ranged from 0.4 to 2.1 mol/$mol_{glucose}$ related to initial pH from 7 to 5.5, respectively. In addition, HY obtained by Xiao et al. [23] was 117.1 ml/$g_{protein}$ at incubation pH of 7 and base pre-treatment at pH of 12. Yang et al. [24] found that HY was 53.0 ml/g_{VS} from lipid extracted micro-algal biomass residues (LMBRs) with thermo-alkaline pretreatment in batches.

3.3. Metabolite by-product components

The profiles of acetate (HAc), butyrate (HBu), propionate (HPr), and lactate (HLa) are summarized in Fig. 3. From a hydrogen production perspective volatile fatty acids (VFAs), HAc and HBu, are the desirable soluble products since hydrogen generation occurs via those reactions [25]. The domain volatile fatty acid component in glucose batches was HBu followed by HAc. However, HAc, HBu, HPr and HLa concentration increased from 0.36 ± 0.07 to 1.81 ± 0.16; from 1.46 ± 0.09 to 3.48 ± 0.12; from 0.14 ± 0.03 to 0.62 ± 0.08 and from 0.45 ± 0.12 to 0.70 ± 0.19 g/l when inoculated glucose with seeding sludge,

respectively. Furthermore, these metabolite products were somewhat increased a little to 2.09 ± 0.23, 3.61 ± 0.09, 0.73 ± 0.06 and 0.77 ± 0.22 g/l, respectively with papaya enzymes supplementation.
On the other hand as depicted in Fig. 3, the mainstream VFAs component in protein batches were HAc followed by HPr. Protein in conjunction with mixed cultures bacteria increased HAc, HBu, HPr and HLa to 1.85 ± 0.19, 0.63 ± 0.11, 1.07 ± 0.16 and 0.18 ± 0.08 g/l compared to 0.98 ± 0.17, 0.29 ± 0.10, 0.91 ± 0.14 and 0.05 ± 0.03 g/l for digested protein by its own (without seeding sludge addition). Addition of *C. papaya* to the aforementioned mixture reformed HAc, HBu, HPr, and HLa to 3.63 ± 0.13, 1.18 ± 0.15, 0.44 ± 0.13, and 0.26 ± 0.03 g/l, respectively. Consequently, the metabolic pathway shifted to produce lower propionate and higher acetate and butyrate. According to the VFAs analysis, the superior acids in the final samples of lipids batches were HAc, and HBu. HAc, HBu, HPr and HLa increased from 0.16 ± 0.03 to 0.53 ± 0.07; from 0.08 ± 0.02 to 0.22 ± 0.05; from 0.04 ± 0.01 to 0.19 ± 0.05; and from 0.08 ± 0.02 to 0.11 ± 0.03 g/l respectively by providing inocula to lipid media. However, these metabolite components were further increased to 1.19 ± 0.15, 0.35 ± 0.05, 0.26 ± 0.04, and 0.16 ± 0.05 g/l by enriched enzymes in batches via *C. papaya*. Notably that HAc significantly increased due to *C. papaya* addition, which may due to lipase enzyme stimulation to convert more glycerol and LCFAs to acetate [26].

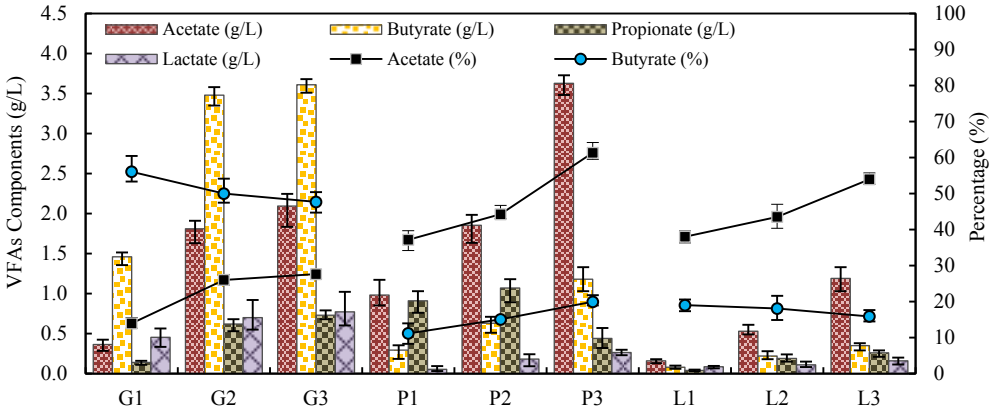

Fig. 3. Soluble metabolites components generated from different batches.

4. Conclusions

The strategy of enzymes enrichment by using *Carica papaya* latex and peels as enzymes source is a promising strategy for increasing HY from protein and lipid. In contrast, it has a negligible effect on glucose. HY from glucose, protein, and lipids reached up to 247.3 ± 23.8 ml/$g_{glucose}$ (1.01-fold), 130.6 ± 8.5 ml/$g_{protein}$ (2.50-fold), and 64.8 ± 3.1 ml/g_{lipid} (1.51-fold) respectively with addition of *C. papaya* compared with seeding sludge supplementation alone.

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Biography

Mohamed Elsamadony is a Ph.D. student at Egypt-Japan university of science and technology with interests in sanitary work, waste treatment, and biohydrogen production. He holds a master's degree in optimization of water network from Menoufia University and a B.A. in structural engineering from Tanta University.