Enrich waste activated sludge digestibility via natural enzyme supplementation

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Abstract. Upgrading of low biodegradable waste activated sludge (WAS) accomplished through supplement the hydrolysis step with natural enzymes source. Whereas, WAS is rich in particulate fractions in terms of total chemical oxygen demand (tCOD), total suspended solids (TSS) and volatile suspended solids (VSS) of 15.78, 14.92 and 12.15 g/L, respectively. Therefore, carica papaya enzymes were utilized to break down the peptide ponds in protein molecules such as papain and protease, as well as, lipases that catalyzed the degradation of lipids. The optimum mixture between papain, protease, and lipase enzymes was found to be 3: 1: 2 while the optimum enzyme concentration was 8%. This conditions was attributed to enhance the H_2 productivity form WAS by 97.8%

1 Introduction

Large amount of wastewater generated daily from municipal and industrial activities which requires a proper treatment through physical and biological process. In this concern, abundant waste activated sludge (WAS) accumulates from secondary sedimentation tank located at wastewater treatment plants [1–3]. The classical technologies for stabilizing and managing this waste amounted about 40 to 60% of the operational cost, because the complex structure of particulate organic fractions originated at WAS [4]. Anaerobic digestion (AD) process represents an interesting choice to get rid of such a waste with add value in term of renewable energy source [5–8]. Whereas, AD has the ability to convert organic waste to energy carrier gases i.e. hydrogen and methane gases [9–14].

However, WAS is rich with protein and lipids fractions which that forms about 80% of the particulate organic fractions [15]. Therefore, pretreatment methods were applied on WAS prior to introduce to AD process to increase its solubility and make it easy to anaerobes to feed on [6]. One of the most common methods is the enzymatic hydrolysis, since it has the ability to reduce the lag phase, enhance the WAS digestibility and improve the liberation of extracellular polymeric substances (EPS) [16]. This is because enzymes such as amylase, protease and lipase degrade the complex polymeric substance and facilitate its transportation to subsequent acidogenesis microorganisms [17]. Protease and lipase producing microorganisms such as *Bacillus sp.* and *Acinetobacter calcoaceticus*, respectively revealed a decline of lipid concentration by 99% after twelve days when treating lipid rich wastewater under aerobic condition [18]. Moreover, the supplementation by lytic enzymes exhibited a COD degradation of 87% of pot ale residues via AD process. This was not the case when no enzymes were added, since only 13% were achieved [19].

On the other hand, commercial enzymes are too expensive which obstacle the practical application of the enzyme use at WAS pretreatment. Therefore, the aim of this study was to explore the availability of using a nature source to extract essential enzymes for WAS solubility such as papain, protease and lipase enzymes. This was accomplished by using Carica papaya plants. Furthermore, single and combined effect of the extracted enzymes on WAS hydrolysis were assessed. In addition, the concentration of the optimum enzymes mixture was tested and the maximum hydrogen potential was evaluated.

2 Material and methods

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2.1. Waste activated sludge and enzymes extracted

Waste activated sludge (WAS) was collected from wastewater treatment plant located at Tanta city from the secondary sedimentation tank. The characteristics of the used WAS were as follows: pH, tCOD, sCOD, TSS and VSS equal to 6.82, 15.78, 0.93, 14.92 and 12.15 g/L, respectively. Papain (Pn), protease (Pt) and lipase (Lp) enzymes were extracted from the latex and peels according to methods explained earlier at the following [20–22].

2.3. Experimental setup

Three batch assays were carried out to estimate sing and mixed enzymes on WAS hydrolysis, as well as, determining the optimum concentration of the ideal mixture. 100 mL WAS were loaded to 250 mL serum bottles then loaded with enzymes according to procedure listed at Table 1. Afterwards, the bottles were purged with N_2 gas to maintain anaerobic condition at cultivated at mesophilic condition (35 °C).

Table 1.
Batch anaerobic experiments of single and mixed enzymes effect.

Exp. 1: Single enzyme effect	Exp. 2: Mixed-enzymes effect with fixed concentration 3% (w/w)	Exp. 3: Effect of enzymes mixture of Pn : Pt: Lp (2:1:3) concentrations
No enzyme addition	Pn : Pt: Lp (2:1:1)	1% (w/w)
3% (w/w) Pn	Pn : Pt: Lp (1:2:1)	2% (w/w)
3% (w/w) Pt	Pn : Pt: Lp (1:1:2)	4% (w/w)
3% (w/w) Lp	Pn : Pt: Lp (3:1:1)	6% (w/w)
	Pn : Pt: Lp (1:3:1)	8% (w/w)
	Pn : Pt: Lp (1:1:3)	10% (w/w)
	Pn : Pt: Lp (3:2:1)	15% (w/w)
	Pn : Pt: Lp (1:3:2)	20% (w/w)
	Pn : Pt: Lp (2:1:3)	

2.4. Analytical methods

Total suspended solids (TS), volatile suspended solids (VS) and chemical oxygen demand (COD were quantified according to APHA [28]. Cumulative biogas was amounted using displacement method. Besides, H_2 content in the eveloved biogas was determined by a gas chromatograph (GC) using method mentioned at [10].

2.5. Solubilization efficiency (%) and Hydrolysis coefficient

Solubilization efficiency of COD_t was obtained to assess the enzymatic hydrolysis effect. The COD solublization was calculated using the following equation, where, α is solubilization efficiency (%)

$$\alpha = \frac{\text{Hydrolyzed COD}_s - \text{Influent COD}_s}{\text{Influent COD}_t - \text{Influent COD}_s} \quad (1)$$

Hydrolysis of organic polymers is often described by a first-order kinetic model, Where, K_h is the solubilisation rate constant

$$-\frac{dX}{dt} = K_h.X \quad (2)$$

$$\ln X = -K_h.t + b \quad (3)$$

3 Results and discussions

3.1. Single enzyme effect on WAS solubility

Fig. 1a and b revealed the effect single effect of each enzyme type on the WAS solublization efficiency. The maximum solubilization efficiency of 27.6% was recorded when papain enzyme was supplemented with concentration of 3% followed by lipase and protease enzymes with percentages of 18.5 and 13.6%, respectively. This is because papain is a strong protein enzyme degrader and more than 60% WAS's organic content is complex protein fraction; therefore, papain enzyme achieved the maximum solublization efficiency [17].

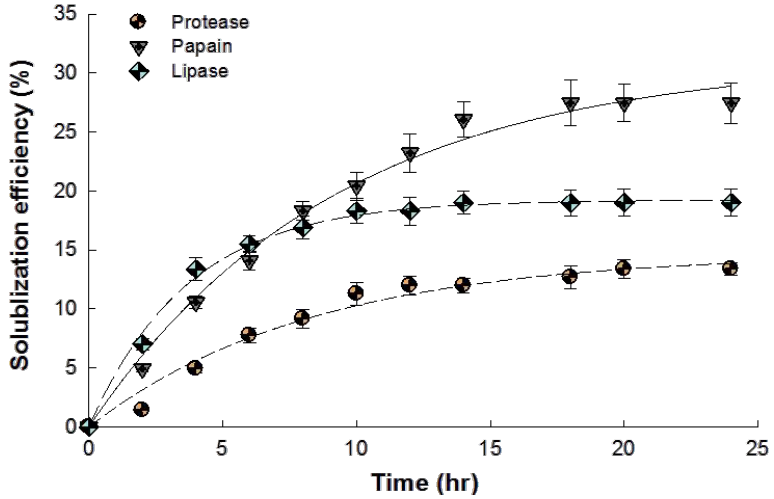

Fig. 1. Solublization efficieny (%) of WAS with single enzyme supplementation.

3.2. Mixed Enzymes effect on WAS solubility

In order to evaluate the synergistic effect of mixed enzymes supplementation, different mixtures of the three enzymes were examined. The optimum mixture was papain: protease: lipase enzymes ratios of 3:1:2. This mixture registered the highest solubilzation efficiency and hydrolysis rate constant of 47.2% and -0.03 h^{-1}, respectively. The synergistic effect between different enzymes were previously emphasized by Yang et al. [24], since they discovered that a significant reduction at VSS content was recorded when use a mixture 1 : 3 of amylase to protease exceeded the single effect of each of them.

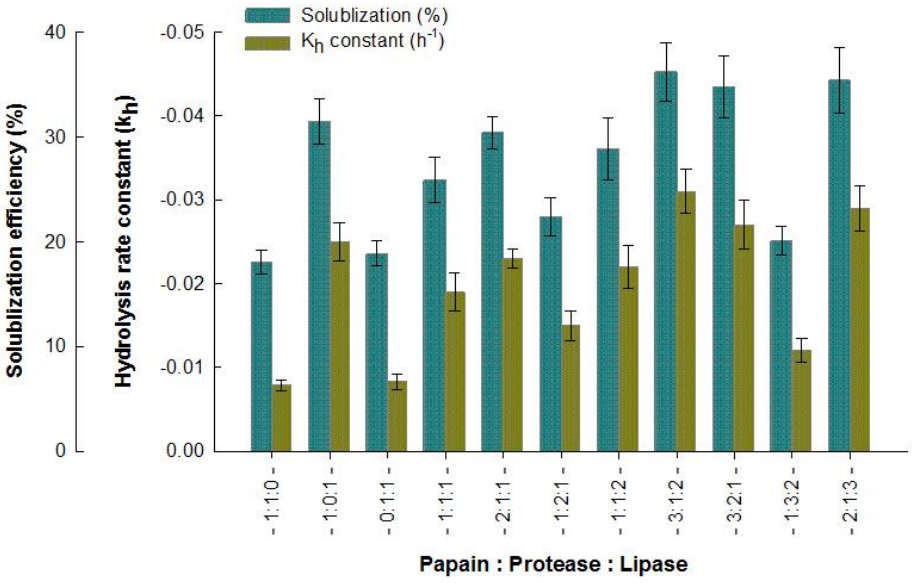

Fig. 2. Solublization efficieny (%) and hydrolysis rate constant of WAS under mixed enzyme supplementation.

3.2. Effect of enzyme concentration on WAS digestibility and H_2 productivity

Solublization efficiency found to be enzyme concentration dependent as explored from Fig. 3. However, after certain concentration a slight increase at solublization efficiency occurred. Similarly, hydrolysis rate constant were achieved the same trend. Maximum solublization efficiency was 52.6% at enzymes concentration of 20%, however, at concentration of 10% solublization efficiency was 47.9%. This means that with doubling the concentration, the solublization efficiency only incremented by 4.7%. On the other hand, H_2 potential were evaluated at different enzyme concentration, maximum H_2 productivity of 452.1 mL were noticed at enzyme concentration of 8% compared with only 228.6 mL at control batch (no enzymes were added). This is due to increasing the solubility of WAS structure, as well as, breaking down the complex compounds to simple compounds that anaerobes able to feed on and convert them into hydrogen. However, further enzyme concentration augmentation led to a reduction at H_2 productivity until reached 179.2 mL at concentration of 20%. This may as a result of enzymes is originally a protein molecule so at certain limit hinder the process, as well as, further dissolution of organics to tiny structure can penetrate the bacterial membrane and kill the anaerobes [16, 25].

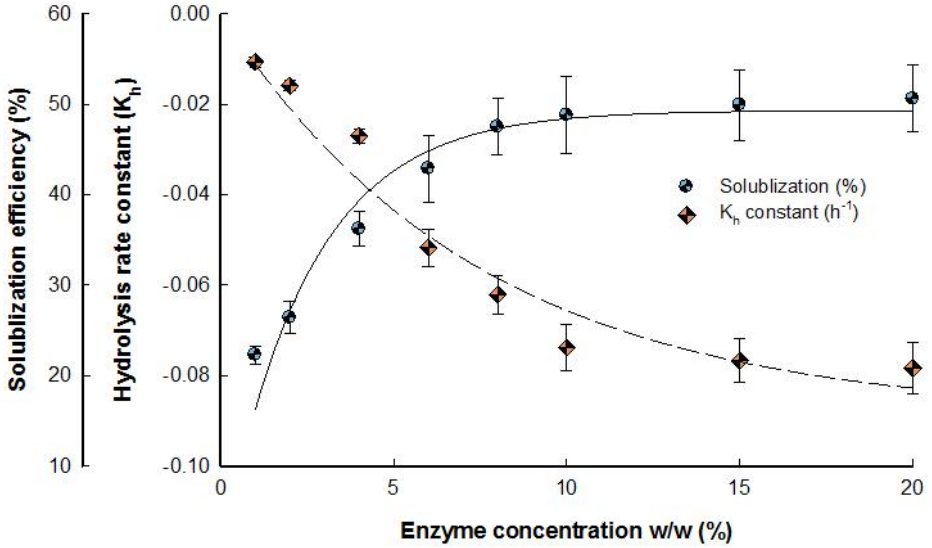

Fig. 3. Solublization efficieny (%) and hydrolysis rate constant of WAS versus enzymes concentration.

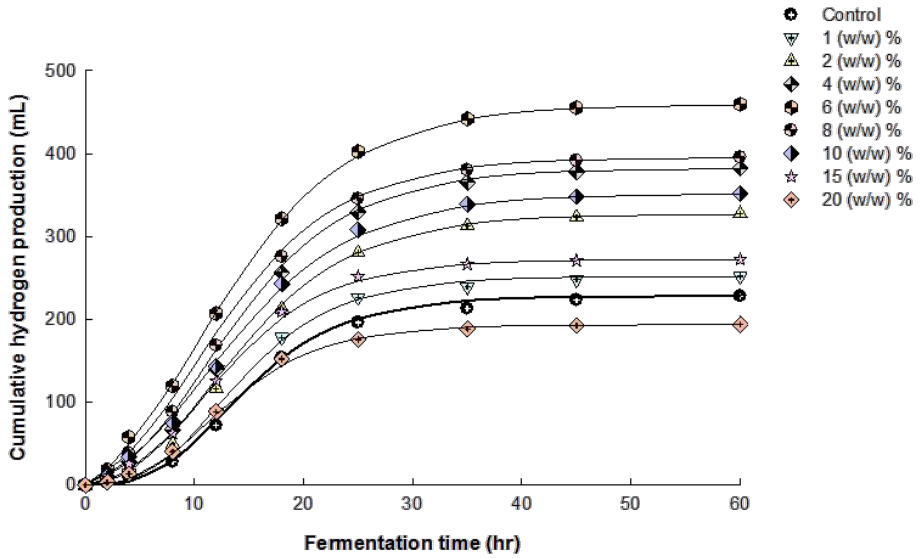

Fig. 4. Hydrogen potential harvested from WAS at different enzymes concentration.

4 Conclusions

Enzymes supplementation found to be an optimistic approach to enhance WAS solubility and improve hydrogen potential. Papain was the most effective enzyme, however, synergistic effect was found between the three tested enzymes with optimum ratio of papain: protease: lipase equal to 3: 1: 2. Moreover, the optimum enzyme mixture concentration was 8% which achieved the highest hydrogen potential of 452.1 mL.

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