Aqueous ammonia soaking pretreatment of spent coffee grounds for enhanced enzymatic hydrolysis

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Introduction

Coffee is among the **top commodities** worldwide, with its **consumption** exceeding **166 thousand 60-kg bags** in 2021. The preparation of a cup of coffee generates various solid residues, including spent coffee grounds (SCG). Over 6 million tons of SCG are being discarded annually.

SCG, primarily a lignocellulosic biomass, could be used to obtain fermentable sugars, that may be subsequently valorized through different bioprocessing routes. However, the composite lignocellulosic structure makes this biomass recalcitrant to biological and chemical biotransformations and the overall downstream processing difficult. Thus, the use of diverse pretreatments (mostly chemical) and other preliminary degradation strategies may facilitate further processing of the SCG residues.

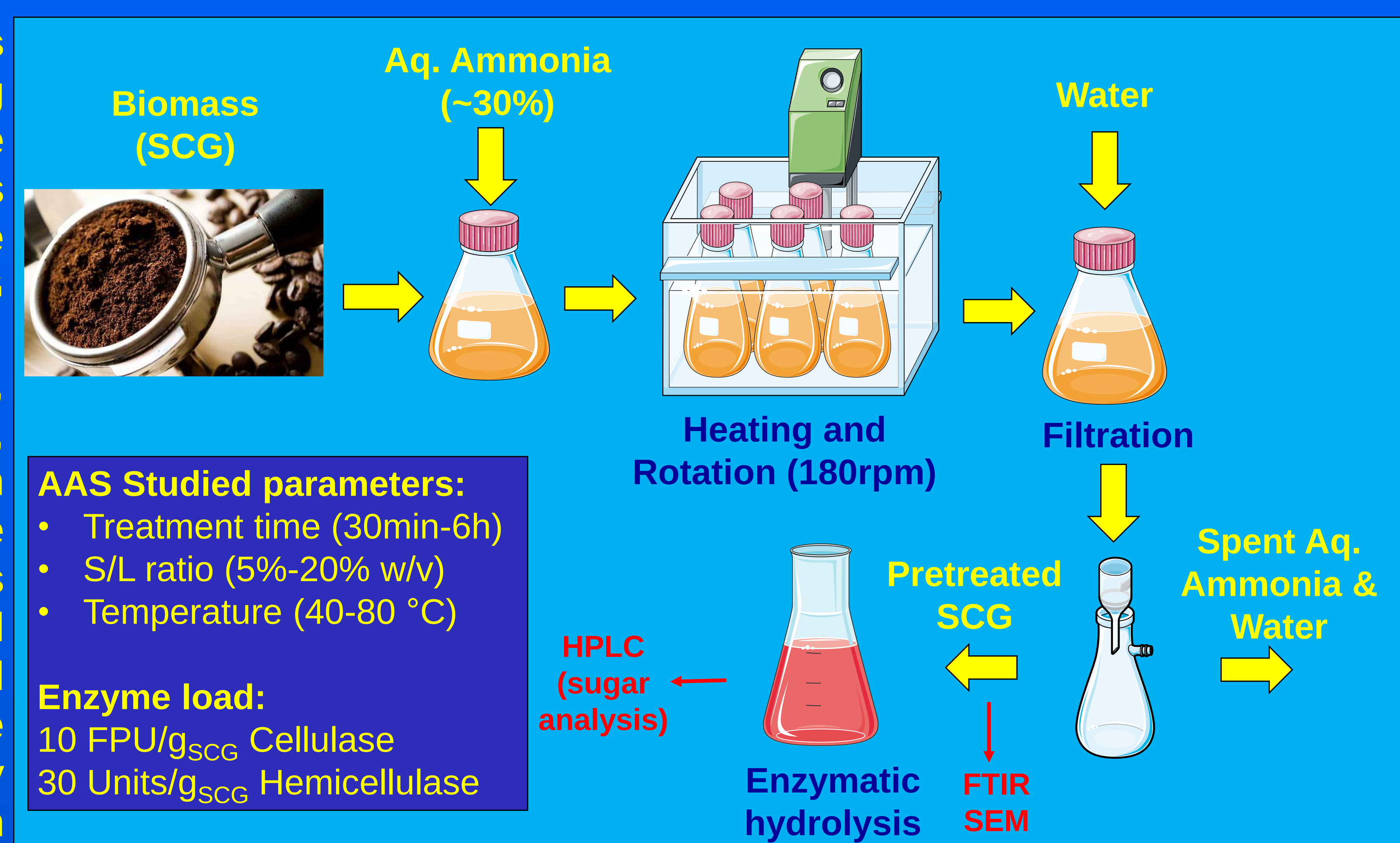

Figure 1: Experimental procedure of SCG valorization

Aqueous Ammonia Soaking (AAS) is lately being considered as an efficient **alternative pretreatment** method for lignocellulosic feedstocks. Aqueous ammonia removes lignin selectively and retains relatively intact both hexose- and pentose-based carbohydrates.

In the present study, AAS was examined as a potential pretreatment stage for the enzymatic hydrolysis of SCG, to obtain fermentable sugars. The studied parameters were **treatment time** (30min-6 h), SCG **solid loading** (5-20% w/v), and **temperature** (40-80°C). Each parameter was studied individually, while keeping the other parameters constant.

Results & Discussion

The rigid surface structure of the untreated lignocellulosic biomass (**Fig. 2**), indicates the need for a pretreatment stage in order to permit an effective enzymatic hydrolysis

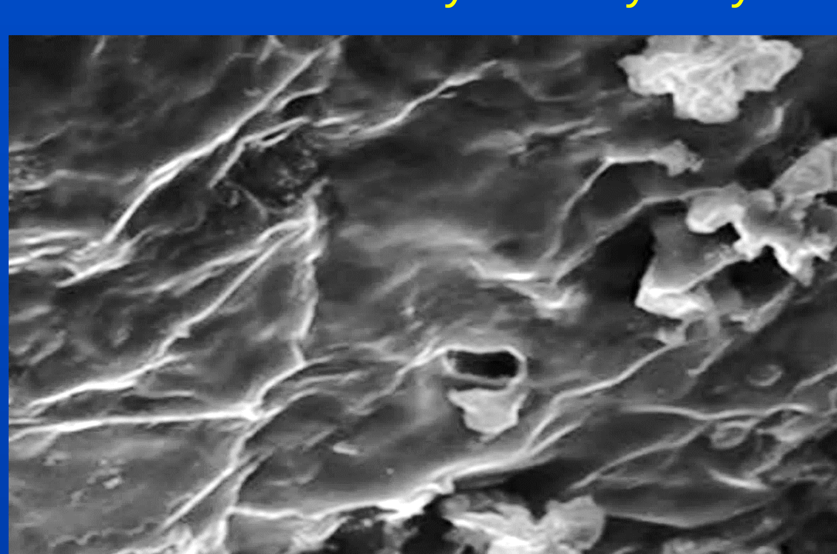

Figure 2: SEM image of untreated SCG

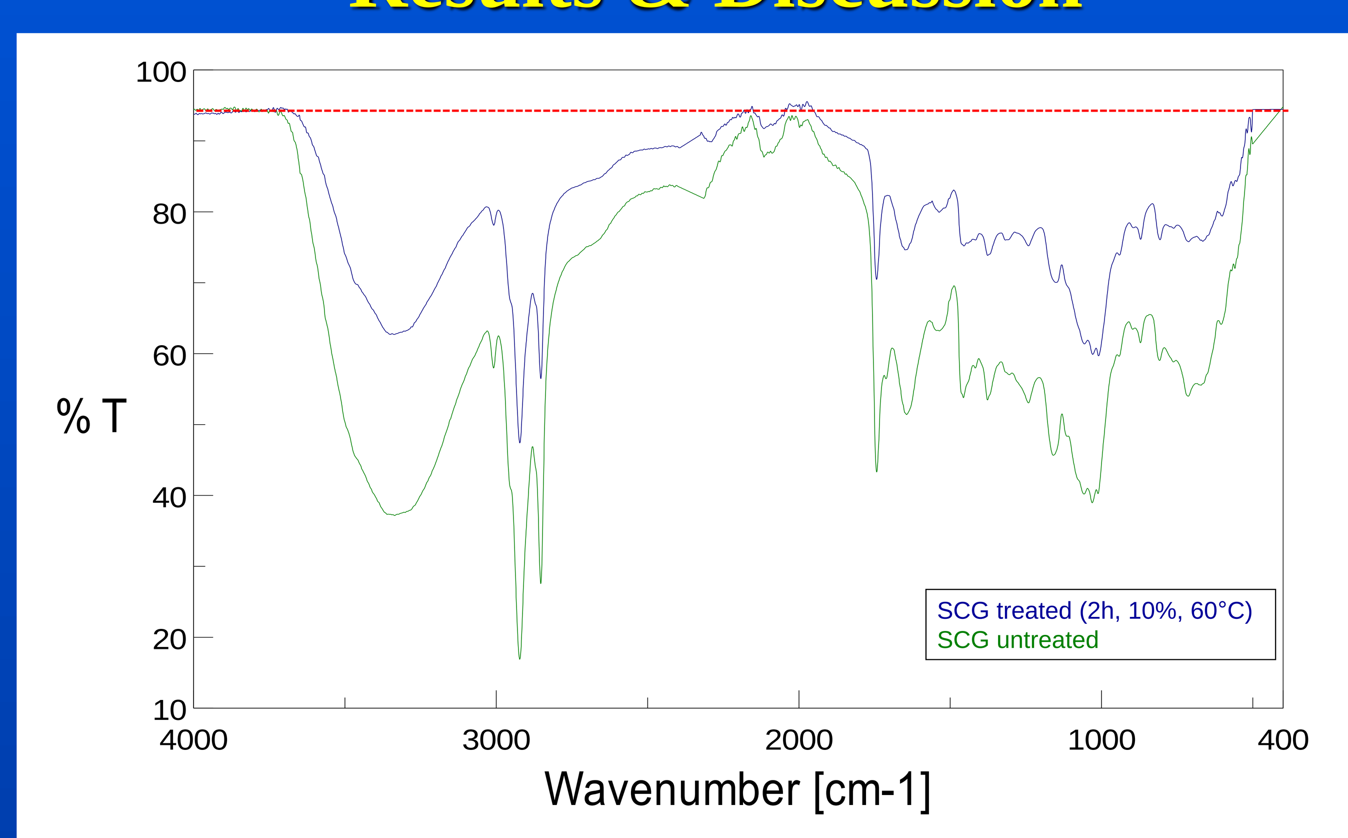

Figure 3: FTIR spectra of AAS treated SCG (2h, 10%, 60°C) and untreated SCG

The FTIR spectra of the SCG samples (untreated and AAS treated) are shown in **Fig. 3**

- The characteristic bands of the functional groups (lignin phenolic residues, chlorogenic acids, caffeine, and hemicelluloses) can be identified in the fingerprint region (1800 - 500 cm^{-1}).
- The reduction of the peak areas throughout the spectra suggests that during the pretreatment process the following matrix changes may occur: (a) intramolecular dehydration reaction; (b) decomposition of the original lignin fraction; and (c) possible rupture of glycosidic linkages of the cellulose and hemicellulose components.

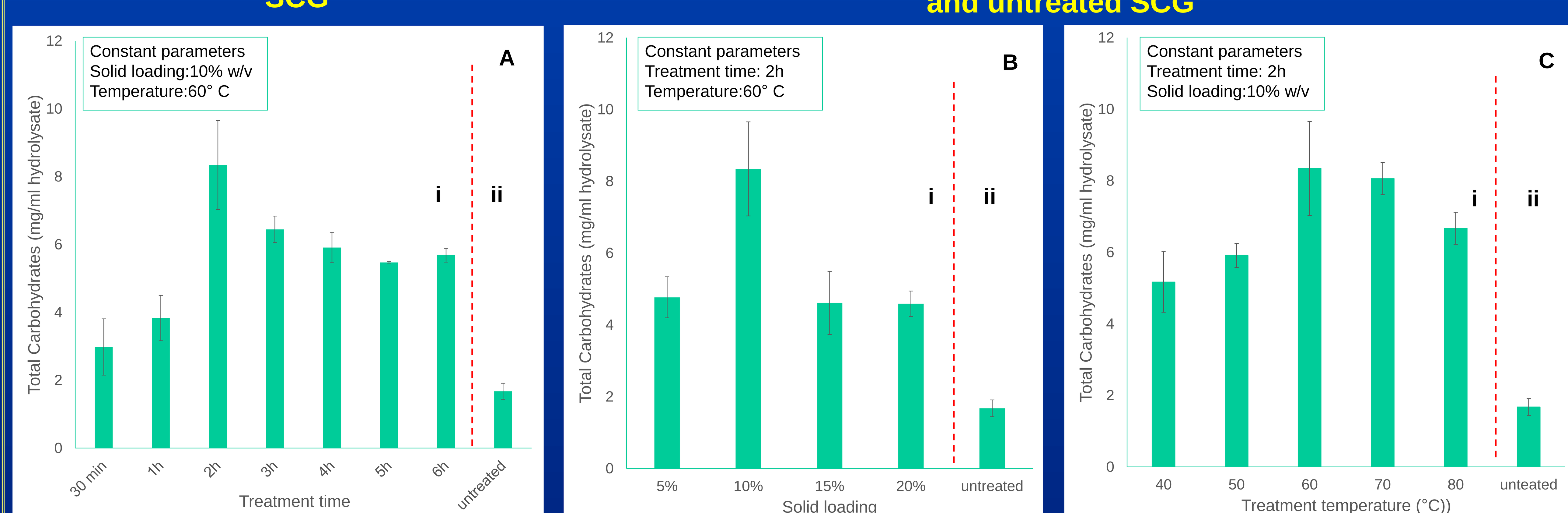

Figure 4: Sugar yield after enzymatic hydrolysis of i) treated samples, ii) untreated SCG *
[A) Effect of Treatment time, B) Effect of SCG solid to liquid ratio, and C) Effect of Temperature of AAS pretreatment]
*Enzymatic hydrolysis results of the untreated SCG have been presented in each graph, for comparisons between the AAS pretreatment and the raw biomass.

- The highest sugar yield (8.34 mg$_{sugars}$/ml$_{hydrolysate}$) after the enzymatic hydrolysis of SCG, was obtained for 2h treatment time with AAS (30 %), at 10% w/v solids loading and 60 °C (Fig. 4 i).
- Increase of the treatment time beyond 2h did not further enhance the final outcome at 10% w/v and 60°C. Similar trends reflecting no further enhancement beyond 10% w/v (Fig 4Bi) and 60 °C (Fig 4Ci), respectively, were also noted.
- Untreated SCG showed the lowest sugar yield (1.67 mg$_{sugars}$/ml$_{hydrolysate}$), emphasizing the need of the AAS pretreatment for improvement of the enzymatic hydrolysis yield under all conditions tested (Fig. 4 ii).

Conclusions

The experimental work presented herein provides one of the first investigations into the potential use of AAS as an efficient method for the pretreatment of SCG. Results showed that AAS had a significant positive impact on the yield of soluble sugars obtained from SCG, after enzymatic hydrolysis, compared to the untreated biomass. An increase of AAS treatment duration beyond 2h (10% solid loading, 60 °C) does not seem to induce greater sugar yields after enzymatic hydrolysis. Furthermore, the solid residue loading of 10% w/v SCG/ Aqueous Ammonia, seems to improve the sugars yield in the enzymic hydrolysates, compared to pretreatments with lower and higher solid loadings (2h, 60 °C); i.e., the former implies an adequate contact between SCG lignocellulosic structures and the solvent. Moreover, with increased pretreatment temperatures above 60 °C there was also no further improvement in the degree of carbohydrate hydrolysis .
Further research is needed to explore the possible interactions among the pretreatment conditions and optimize all parameters involved, in order to properly design a cost-effective process for the valorization of SCG.

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