Acoustic and hydrodynamic cavitation assisted hydrolysis and valorisation of waste human hair for the enrichment of amino acids

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ABSTRACT

Hair waste in large amount is produced in India from temples and saloons, India alone exported approximately 1 million kg of hair in 2010. Incineration and degradation of waste human hair leads to environmental concerns. The hydrothermal process is a conventional method for the production of hair hydrolysate. The hydrothermal process is carried out at a very high temperature and pressure, which causes the degradation of heat-sensitive essential amino acids, thereby depleting the nutritional value. This work deals with alkaline hydrolysis of human hair using acoustic and hydrodynamic cavitation, and comparison with the conventional method. The optimal operating conditions for highest efficiency was observed, for the hydrolysis of 1 g of sample hairs in 100 mL of solution, at 4:1 (KOH: hair) ratio, soaking time of 24 h, the ultrasonic power density of 600 W dm^{-3} (20 KHz frequency and input power 200 W) or hydrodynamic cavitation inlet pressure of 4 or 7 bars. Cavitation results in rupture of disulfide linkages in proteins and mechanical effects lead to cleavage of several hydrogen bonds breaking the keratin sheet structure in hair. Breakdown of bonds leads to a decrease in viscosity of the solution. 10% and 6% reduction in viscosity is obtained at optimal conditions for ultrasonic and hydrodynamic cavitation treatment, respectively. FTIR analysis of produced hair hydrolysate confirmed that the disulfide bonds in hair proteins are broken down during cavitation. The amino acid of hair hydrolysate, prepared using cavitation, has a relatively higher digestibility and nutritional value due to the enhancement of amino-acid content, confirmed using amino acid analysis. Cavitation assisted hair hydrolysate has a potential application in agricultural engineering as a fertilizer for improvement of the quality of the soil and land. Cavitation based hair hydrolysate can also be used as an environmentally friendly and economical source of essential amino acids and digestibles for animal or poultry feed.

1. Introduction

Human hair waste is produced in a large amount all around the world. India alone exported approximately 1 million kg of human hair and hair products worth $238 million in 2010, and global imports were estimated at $1.24 billion [1,2]. Human hair is often used for applications in fashion, beauty accessories, human hair care products and cosmetic brushes [1]. Landfilling and burning of hair without appropriate treatment is an environmental concern and a potential environmental hazard. The sulphur present in hair leads to toxic fumes on incineration and thermal degradation. Thermal degradation of keratinous wastes can cause a foul smell and extreme discomfort [3]. Thermal degradation also leads to a loss of possible revenue and resources. There is hence a need for optimization and intensification of processes which

are sustainable for the environment and will also reduce hair waste along with generating appropriate revenue.

Human Hair is made up of proteins, lipids, water, pigments and trace elements. Depending upon its moisture content which can be up to 32% by weight, human hair is made up of 65% to 95% proteins [4]. Amino acids that are found in hydrolysates of hair are Alanine, Arginine, Aspartic acid, Cystine, Cysteic, Citrulline, Isoleucine, Glycine, Glutamic acid, Histidine, Leucine, Lysine, Methionine, Ornithine, Phenylalanine, Proline, Serine, Tyrosine, Threonine, Tryptophan, and Valine [5]. Human hair contains approximately 50% by weight carbon, 7% by weight hydrogen, 22% by weight oxygen, 16% by weight nitrogen, 5% by weight sulphur, with slight differences according to the source of the hair; the ash content is of about 0.3–1% by weight [6]. Hair keratins can be classified as α-keratin [7], and these keratinous structures are difficult to degrade because of the high content of cysteine disulfide bonds

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Nomenclature

NUS	Non-ultrasonic (Conventional)
US	Ultrasonic
HC	Hydrodynamic Cavitation
HPLC	High performance liquid chromatography
FTIR	Fourier transform infrared spectroscopy
C_v	Cavitation Number
P_v	Vapour pressure
V_{th}	Velocity (m s^{-1}) at the throat of venturi
r	Density of the solution
P_2	Fully recovered downstream pressure

[8].

Human hair has been used as a bio adsorbent using a chemical treatment for the uptake of phenol [9], which can be used in place of activated carbon [10]. Hair, without chemical treatment, can also be used for bioadsorption of heavy metals [11]. Use of hair is often cited in agricultural applications as a fertilizer mainly because of the nitrogen content of hair proteins. Horticultural crops like lettuce and wormwood were studied for application of human hair as a nutrient source fertilizer [12]. Hair waste increased the yield of basil, thorn apple, peppermint, and garden sage by acting as a source of nitrogen nutrient [13]. While the biological decomposition processes take several months [14], human hair can also be decomposed within a few hours by acidic or alkaline hydrolysis at high temperatures and pressures. Amino acids constitute to majority of the hydrolyzed solution and can be used as a liquid fertilizer after neutralization. Keratin from human hair can be converted to l-cystine using acidic hydrolysis which can be used further in fertilizers [15]. It has been reported that this hydrolysate can be used for enhancement of the chlorophyll content and biomass in spinach and wheat plants [16]. Alkaline hydrolysis of hair is used as a source of cysteine as sulphur can be removed from hair in appreciable amounts [17]. Alkaline hydrolysis of hair in the presence of sulphides have shown that it increases the rate of hydrolysis, that the steps involved will be a reaction between hair and sulphide and the reaction between altered hair and hydroxide [18]. Application of hair hydrolysates to soil also demonstrates improvements in the colour, size and growth of *Amaranthus dubius* and hot pepper plants [19]. As for acid hydrolysis, hair's acid combination capacity is approximately 820 μmol/g [5]. Sub-critical water hydrolysis of hog hair was performed for the production of amino acids; the temperature was around 200–300 °C in an high-temperature high-pressure batch reactor at saturation vapour pressure for up to 360 min [20]. Also, acid hydrolysis of hair can be done with HCl with hair weighing from 0.05 to 0.5 g for 48 Hours along with a process using bisdinitrophenyl-cystine for sulphide interchange reaction [21]. This indicates the use of high amount of energy in the processes. The energy consumption needs to be reduced in hydrolysis procedures to make the process economical. Hence, the application of cavitation for the process intensification of hair hydrolysis is suggested. Cavitation based hair hydrolysis can be performed at room temperature, this avoiding the use of high temperature and high-pressure conditions.

Cavitation is marked by the violent collapse of bubbles in the liquid medium. This collapse gives rise to a localised high-pressure and high-temperature condition which consequently has various chemical and physical effects. Cavitation can be produced using ultrasonic techniques (Acoustic Cavitation) [22,23], or by hydrodynamic techniques [24–26]. One of the useful effects includes highly reactive hydroxyl ion formation during the collapse of the bubbles [27,28]. According to a recent review, human hair hydrolysis, with or without cavitation, has never been studied for chemical changes during hydrolysis and enhancement of amino acids during the process [29].

The main aim of this work is to study the effect of cavitation (acoustic and hydrodynamic) on the process of hydrolysis of hair, chemical properties of human hair, and further, it's comparison to the conventional method of hydrolysis in an autoclave. This hydrolysate can be potentially used as a fertilizer for agricultural applications- improving the soil quality, thereby reducing the use of synthetic fertilizers in Indian agriculture and can be used as an animal feed for providing nutritional amino acids. The use of this process will have economic as well as environmental benefits.

2. Experimental

2.1. Chemicals and reagents

Waste Human Hair was collected from local hair salons in Mumbai and was used as received without any chemical purification. Reagent grade potassium hydroxide, Sodium hydroxide, ortho-phosphoric acid and HPLC grade Sodium acetate, acetonitrile, methanol, and phenyl isothiocyanate were purchased from SD Fine Chemicals Ltd. Mumbai. Ultra-pure D.I. water was prepared using Millipore apparatus. All reagents were used as received without further purification. All experiments were performed at room temperature (30 °C).

2.2. Non-ultrasonic treatment (NUS)

Waste hair was finely cut into a length of 1 mm or less for further use. Different concentrations of 100 mL alkaline solutions were prepared with the ratio of KOH: NaOH as 14:1. Concentrations of KOH used for the hydrolysis were 0.5, 1, 2, 3, and 4% (weight to volume). 1 g of hair was dissolved in each of these solutions for the first run. Non-ultrasonic (NUS) dissolution of hair was done by preparing the above hair solution and soaking at 140 rpm at 30 °C for 24 h. The sample was filtered using whatman® filter paper 1 and dried in an oven at 100°Cfor 48 h, and percentage hydrolysis was calculated. All the runs were repeated for consistent results.

$$\%Hydrolysis = \frac{Initial\,dry\,hair\,weight - dry\,filtered\,cake\,weight}{Initial\,dry\,hair\,weight} \quad (1)$$

As a continuation of the NUS method, the solution after the initial dissolution was processed at 5 bars and 120 °C for 15 min in an autoclave. This was done for a comparison of the conventional method (NUS) with treated samples for HPLC and FTIR analysis.

2.3. Ultrasonic treatment (US)

Different concentrations of hair in 100 mL solutions were used for the second run (0.25–3 W/V %). Ultrasonic (US) treatment was performed with 50 W power input (150 W dm^{-3} power dissipation) after the initial dissolution for 24 h (Fig. 1). US irradiation runs were performed using ultrasonic horn operated with 5 s ON and 5 s OFF pulse based on both manufacturer recommendations and our previous reports to avoid excessive heating and damage to the transducer [23]. The ultrasonic horn (M/s Dakshin, Mumbai) had a fixed operating frequency of 20 kHz, tip diameter of 2.1 cm and maximum rated power dissipation of 240 W. The optimised hair and KOH concentration was taken for evaluating the effect of power dissipation (50 W, 100 W, 150 W and 200 W). Temperature was controlled using a continuous flow ice-bath, temperature fluctuations were within ± 3 °C. The efficiency of the ultrasonic horn was calculated to be approximately 30% for the tested range of input power by calorimetric method [30]. The efficiency for the horn agrees well with recently reported calorimetric-derived efficiencies for the same horn [31].The efficiency of ultrasonic treatment was measured by the relative decrease in viscosity with the progress in ultrasonic irradiation. The relative viscosity of all the solutions was measured by Ubbelohde glass capillary viscometer. Time was measured using a stopwatch with a maximum error of ± 0.01 s. All viscosity measurements were repeated at least 3 times for precision. All data presented
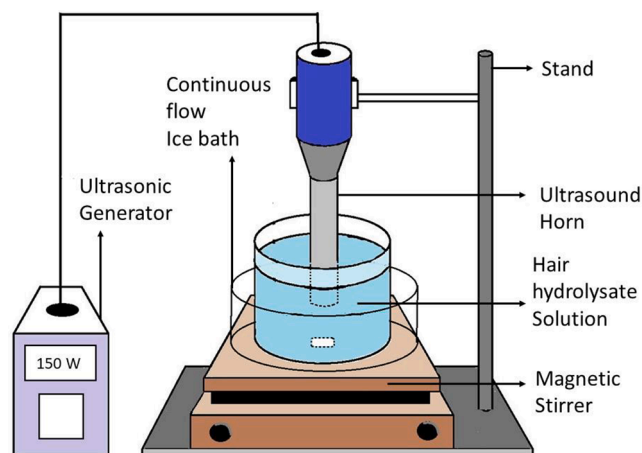
Fig. 1. Experimental setup for ultrasonic cavitation (US) treatment. The horn is dipped approximately 2–3 cm inside the continuously stirred hair solution.

have error bars that indicate standard deviation around the mean. Further, the percentage reduction in viscosity was measured with respect to the initial viscosity. Additionally, 2% (V/V) basis of isopropyl alcohol was added in an experimental run with 200 W input power and 1% hair as a scavenger run.

2.4. Hydrodynamic cavitation treatment (HC)

Solutions for hydrodynamic cavitation were prepared using 4% (W/V) KOH and 1% (W/V) of waste hair. Hydrodynamic cavitation treatment was run for 30 min post the initial dissolution for 24 h. Variation in viscosity every 10 mins was evaluated for different pressures from 4 to 7 bars. The hydrodynamic cavitation setup consisted of a tank with 5 L alkali-hair hydrolysate solution (Fig. 2). Venturi was used as the cavitating device as used in our previous studies [30,31]. Bypass valve V2 was used to control and vary the pressure at the inlet of the venturi (P1). V3 was completely open during all runs. Flowrate at the outlet of the

venturi was calculated for determining the number of passes and real cavitation irradiation time.

2.5. Analysis

The final solution in all runs was prepared by the subsequent addition of ortho-phosphoric acid to prepare fertilizers for analysis. The final solutions were freeze-dried. Further, the samples were analysed using Fourier transform infrared spectroscopy (FTIR) and High-Pressure Liquid Chromatography (HPLC) for amino acids.

2.5.1. Fourier transform infrared spectroscopy
FTIR analysis was performed for NUS and US samples to analyse the mechanistic changes happening to the structure and bonds following the results obtained from our previous research [28]. FTIR analysis was performed using SHIMADZU IR Prestige 21. The changes in percentage transmittance were obtained for the mid-IR region of 400–4000 cm^{-1} with a 16 scan-speed. FTIR was only used for NUS and US to study the qualitative changes in hair sample principally.

2.5.2. High-performance liquid chromatography
HPLC was performed using reverse phase HPLC 1200 series by Agilent Technologies. Buffer A was prepared as 10 mM Sodium acetate, pH6.4 with 6% acetic acid, and buffer B was prepared by mixing A buffer with Acetonitrile (40:60). 1 mg sample was dissolved in 1 mL of D.I. water and further adjusted to 5 mL using HPLC grade methanol. The solution was incubated at −20 °C overnight, centrifuged and the supernatant was evaporated using nitrogen gas bubbling at 60 °C. The sample was added to 50 μL of phenyl isothiocyanate reagent and placed on thermo mixer for 1 h at 45 °C. After vacuum drying, 200 mL buffer A was added and centrifuged. The supernatant was filtered using a syringe filter. 20 μL sample was further taken for HPLC analysis. The flow rate was 1 mL/min, and detection was done at 254 nm. The run was started with 100% A; at 25 min changed to 87% A and 13% B; at 68 min changed to 45% A and 55% B; at 70.5 min changed to 100 %B and at 78 min changed to 100% A till 82 min.
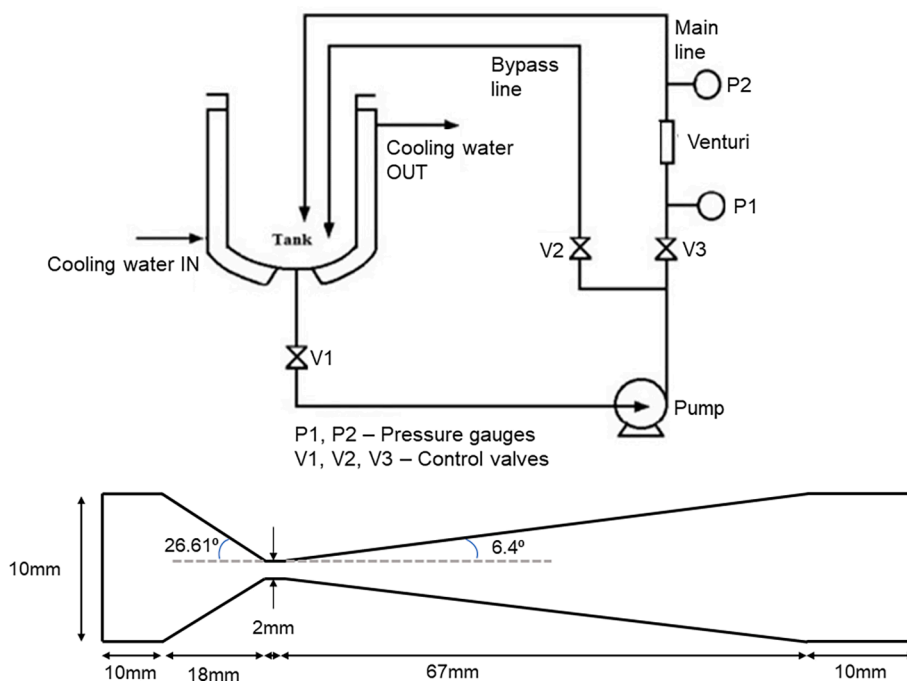

Fig. 2. Experimental setup (top) for hydrodynamic cavitation treatment with venture obstruction. Geometry and dimensions of the venturi (bottom) used in the setup.

3. Results and discussion

3.1. Non-Ultrasonic hydrolysis

Initially, the concentration of KOH for alkaline hydrolysis was optimized using varied KOH concentrations of 0.25 to 5% (Weight to Volume basis). Hair weight was held constant at 1 g (1% Weight by Volume). The amount of hydrolysis increased almost linearly with the KOH concentration up to 3% KOH, followed by a sudden flattening of the plot (Fig. 3). The maximum hydrolysis was observed to be 90.5%. Presence of KOH leads to the weakening of poly-peptide linkages in hair, leading to alkaline dissolution. The higher presence of KOH is observed to yield higher dissolution of hair up to a threshold, following which there is no observed increase in hydrolysis. KOH concentration of 4% (Weight to Volume) was chosen as an optimum value for hydrolysis of 1% hair sample for further investigation. An experimental run with higher mixing speed of 350 rpm as opposed to 140 rpm was performed and yielded the same percentage hydrolysis value for 4% KOH concentration, and hence all reported speeds of dissolution were 140 rpm.

For determining optimum hair concentrations for efficient hydrolysis, various concentrations of hair loading between 0.25 and 3 % were used with alkali:hair ratio held constant at 3:1 i.e. at lower hair loading, alkali loading will be equivalently low. The extent of hydrolysis and hair dissolution peaks were between 1 and 2% hair loading (Fig. 4). There is a drop in the hydrolysis on either side of the peak. The reduction in the extent of hydrolysis for hair concentrations above 2% is due to aggregation of hair at high loadings. This leads to decreased alkali accessibility to poly-peptide bonds and thereby reduced hydrolytic coverage. At substantially lower concentrations of hair loadings (<0.5%), the equivalent alkali loading also remains low leading to reduced availability for hydrolytic coverage due to lower alkali presence. An optimum exists at 1–2% hair loading, agreeing with trends reported in the literature for hydrolysis of keratinous substances.

3.2. Ultrasonic (US) treatment

Ultrasonic treatment is known to increase the hydrolysis further because the accessibility to disulfide bonds increases due to the mechanical effects of ultrasonic irradiations [28]. The extent of hydrolysis calculated using eq. (1) resulted in 100% hydrolysis for all samples. Additionally, As ultrasonic treatments were performed, the viscosity of the solutions went on decreasing. This was due to improved hydrolysis, and chemical changes that occurred in the solution during the ultrasonic irradiations. Fig. 5 shows the effect of ultrasonic treatment on the
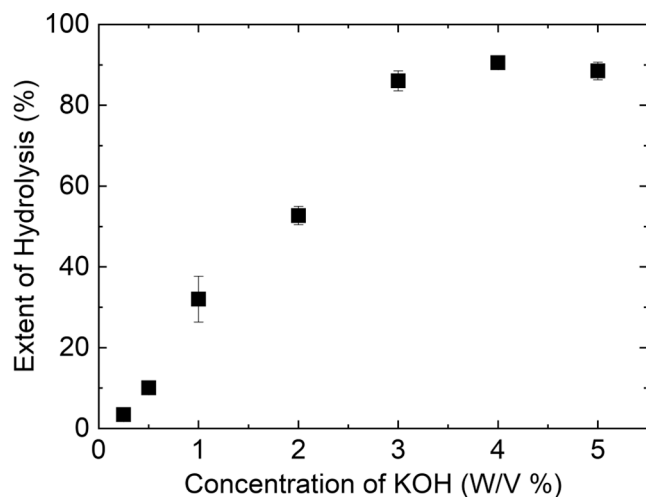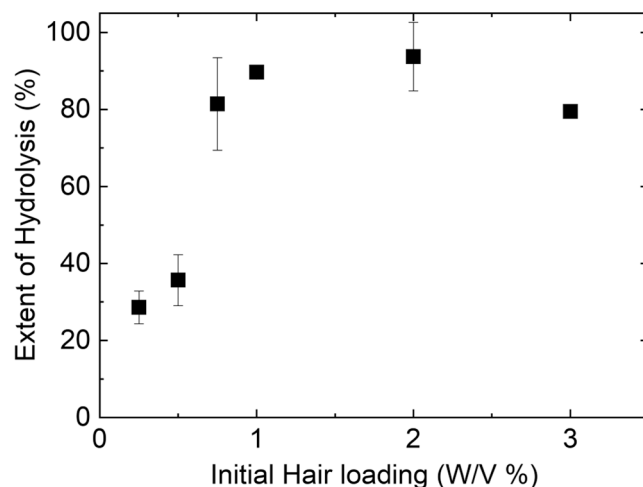

Fig. 4. Effect of hair loading on the extent of hydrolysis for different initial hair loadings.
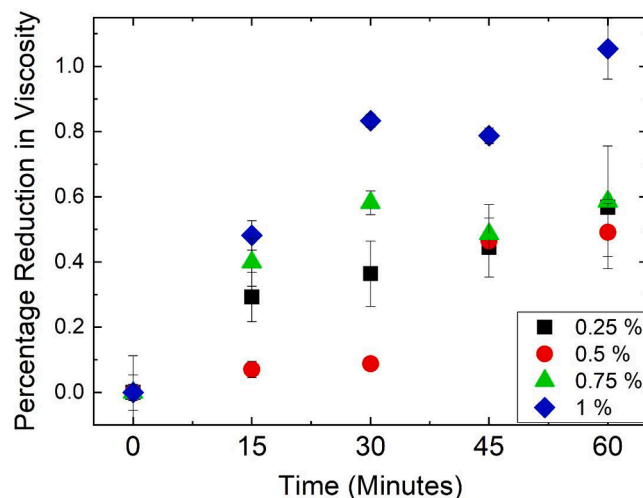

Fig. 5. Percentage reduction in viscosity of hair hydrolysate solution at different hair loadings. Note that each hair loading (0.25 to 1%) yielded a different initial viscosity due to the concentration of hair present. The figure shows a reduction in viscosity of each sample respective to its initial viscosity.

percentage reduction in viscosity for different hair loadings. Time scale shows the total time (ON time + OFF time) for the ultrasonic horn. 1% of Hair loading resulted in the highest reduction in viscosity. Reduction in viscosity increases as hair loading increased to 1%. The extent of viscosity reduction is directly dependent on the amount of hair present. At substantially lower concentrations of hair loadings (<0.5%), the equivalent alkali loading also remains low leading to reduced availability for hydrolytic coverage due to lower alkali presence, leading to lower viscosity reductions. Viscosity reductions are attributed to both mechanical perturbations and chemical changes occurring due to cavitation [32–35]. Chemical changes occurring during cavitation includes the in-situ generation and successive attack of free hydroxyl radicals on the disulfide and poly-peptide bonds in the hair fibre. Depolymerization has been well established to occur as a consequence of cavitation in various studies leading to a decrease in strength of the fibre and subsequent rupture of polymer bonds [33,35–38,40]. To further investigate the reduction in viscosities, the power dissipation during ultrasonic irradiations was increased.

Power dissipation and rating is the most crucial parameter during cavitation processes induced by ultrasound. To further investigate the

Fig. 3. Effect of concentration of KOH on the extent of hydrolysis for 1% (W/V) Hair concentration.

reduction in viscosities, the power dissipation during ultrasonic irradiations was increased. The higher extent of reduction in viscosities was observed with changes in ultrasonic power dissipation (Fig. 6). Up to 9.6% reduction in viscosity could be achieved at the maximum ultrasonic power dissipation of 200 W. Reduction in viscosities indicates strong chemical changes in the hair solution. At lower power dissipations; 4.8% maximum viscosity reduction was observed at 150 W, 3.4% at 100 W and 2% at 50 W respectively. Reduction in viscosity and subsequent chemical changes related to the reduction in viscosity is directly related to the power dissipation. Higher power dissipation leads to both, stronger mechanical perturbations and greater extent of radical generation and cavitational effects, and its impact on hair hydrolysis. 200 W power input (power density of 600 W dm^{-3}) is the optimum power rating for ultrasonic hydrolysis of waste hair.

The analysis of mechanism and effect of ultrasound was performed using isopropyl alcohol (IPA) as a hydroxyl radical scavenger (Fig. 6 B). The viscosity reduction in the initial 15 min for both solutions (with and without IPA) reveal that the mechanism is not hydroxyl radical mediated. Mechanical effects of ultrasound caused due to sheer forces and microstreaming events during cavity implosion lead to this initial decrease in viscosity. It is possible that weaker hydrogen bonds are broken due to the mechanical perturbations during sonication. The continuous decrease in viscosity of the solution without IPA as opposed to the solution with scavenger reveals that along with mechanical
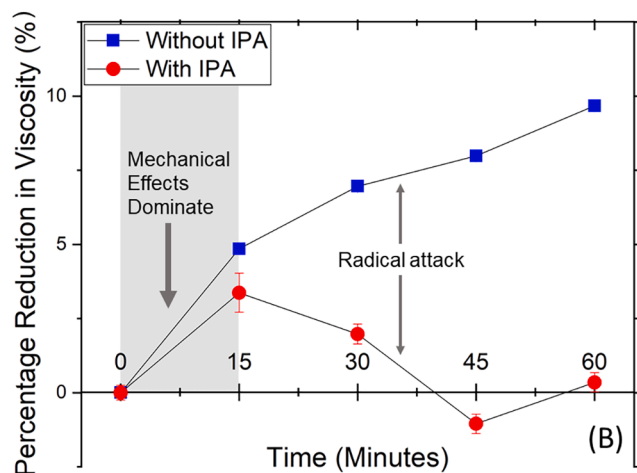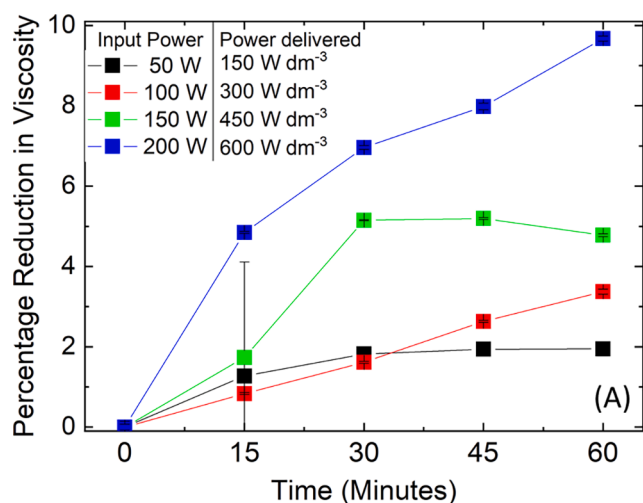
effects, hydroxyl radicals play an important role in the cleavage of bonds beyond 15 min. Discrete distinction between chemical and mechanical effect of ultrasound is difficult to be evaluated. The reason for the final increase in viscosity reduction of the scavenged solution (red dots, Fig. 6B) after 45 min is possibly due to excessive generation of competition by hydroxyl radical as time progresses.

3.3. Hydrodynamic cavitation (HC) treatment

Analogous to power dissipation in ultrasonic cavitation, inlet pressure in hydrodynamic cavitation is the most important parameter affecting the process [39]. Pressure for the HC runs was changed from 4 to 7 bars (Fig. 7). Results for pressures below 4 bar and above 7 bars are not shown because of no apparent viscosity reduction. From 4 bars to 6 bars, the extent of viscosity reduction reduced from 4.5% to 2.7%. Further by the increase of pressure to 7 bars, the maximum reduction in viscosity jumped to 6%. The efficiency of hydrodynamic cavitation highly depends on the cavitation number [25]. Cavitation number, a dimensionless number, characterizes the flow conditions and cavitating conditions could occur over a wide range of cavitation numbers (usually less than unity) depending on the solution under consideration. C_v is the cavitation number, P_2 is the fully recovered downstream pressure, P_v is the vapour pressure of the solution, V_{th} is the velocity through the constriction and r is the density of liquid [42]. There is a dip in cavitational activity between cavitation numbers 0.18 and 0.13 while C_v of 0.22 and 0.11 yielded a higher reduction in viscosity (Table 1). This is potentially due to the trade-off between the dominance of chemical or mechanical effects of cavitation at lower (4 bar) and higher (7 bar) pressures respectively. In comparison between 4 bars and 7 bars in terms of no. of passes, passes for 7 bar pressure are significantly higher than 4 bar, and hence it is expected that viscosity reduction is also higher. Increasing the number of passes for 4 bar pressure by increasing the circulation time to 45 min, did not yield any further reduction in viscosity. It is possible that the trade-off between mechanical shear forces and chemical changes causes the trade-off observed in viscosity reductions at low and high pressures.

$$C_v = \frac{P_2 - P_v}{2\rho . v_{th}^2}$$

In comparison between ultrasonic and hydrodynamic treatment, with respect to reduction in viscosity, US treatment was better, yielding a reduction of almost 10% compared to a 6% maximum reduction by HC. This presents a trade-off between the two processes. While the

Fig. 6. (A) Percentage reduction in viscosity of hair hydrolysate solution upon Ultrasonic cavitation (US) treatment for various input powers (with their corresponding delivered dower densities) and (B) reduction in viscosity with and without the use of hydroxyl radical scavenger/inhibitor (isopropyl alcohol, IPA) at the input power of 200 W (600 W dm-3 delivered power density).
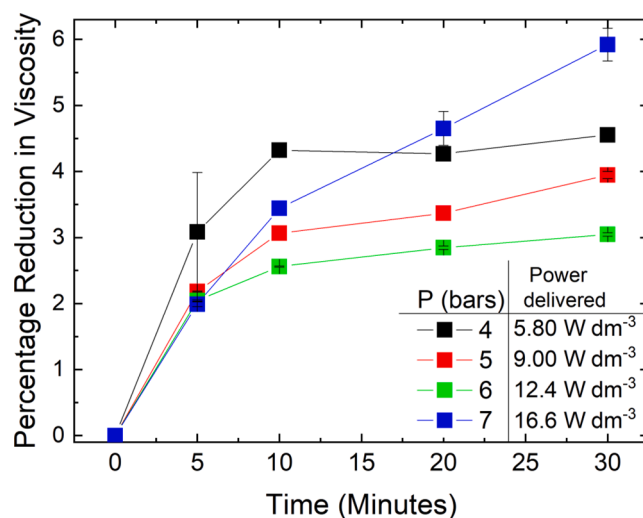

Fig. 7. Percentage reduction in viscosity of hair hydrolysate solution upon hydrodynamic cavitation (HC) treatment for various pressures (P).

Table 1
Cavitation number and volumetric flow rate corresponding to each flow rate for hydrodynamic cavitation.

Pressure (Bars)	Cavitation Number	Volumetric flow rate (LPH)	No. of passes
4	0.22	350	35
5	0.18	405	40.5
6	0.13	450	45
7	0.11	488	48.8

percentage reduction and subsequent chemical changes will be higher in ultrasonic treatment, the cost of ultrasonic treatment remains high due to power usage. HC systems can treat much higher volumes in the same amount of time (5 L in HC compared to 100 mL in the US) as well as at lower costs. Many previous studies have concluded that HC can be used as a pilot-scale treatment and offers great promise with respect to scale up. Since the volume in US reactors is lower, there is higher accessibility of cavitation effect on the pollutant (in this case, hair). This study is also in agreement with the general trade-off observed between cost and efficiency of treatment [41,54].

3.4. FTIR analysis

Disulfide bonds provide mechanical stability to hair and make hair fibers. Disulfide bonds are most susceptible to attack during hydrolysis

and degradation of hair fibres and proteins [44,45]. Cleavage of disulfide bonds has been reported several times as an important step in degradation of keratinous substances and are characterized by high reaction rates [46]. Changes to disulfide bonds in the current study were analysed by FTIR for understanding the mechanism of hydroxyl radical attack on hair (Fig. 8).

Both the spectra show distinctively similar bands between 4000 and 1250 cm^{-1} (Fig. 8). None of the significant changes observed is between wavenumbers 4000 and 1250 cm^{-1}. Transmittance peaks near 3300 cm^{-1} are assigned to N–H stretching vibrations. Keratin transmittance bands fall around 1650 cm^{-1}, 1535 cm^{-1} and between 1300 and 1220 cm^{-1} assigned to Amide I, II, and III, respectively [47]. These bands are in good agreement with keratins found in wool and wool hydrolysates. The behaviour of curves is also in good agreement with the hydrolysis of wool using ultrasonic cavitation [28,29].

The sudden increase in absorbance (decrease in transmittance) between the range of 1220 and 1000 cm^{-1} include the products of cysteine oxidation by-products, including the stretching vibrations of S-O bond in cysteine sulfinic acid [44,45,48]. The strong peak obtained from the curve deconvolution at 525 cm^{-1} is associated with the formation of cysteine thio sulfonate and cysteine thio sulfonic acid, i.e. the stretching vibrations of asymmetric S═O group [48] (Fig. 8).

Long and extended exposure to strong radicals like •OH radicals formed during cavitation can oxidize cysteine bonds (Fig. 9). The most accessible bond for radicals to attack is the disulfide bonds in proteins. Oxidized cysteine loses its ability to absorb electrons formed during a
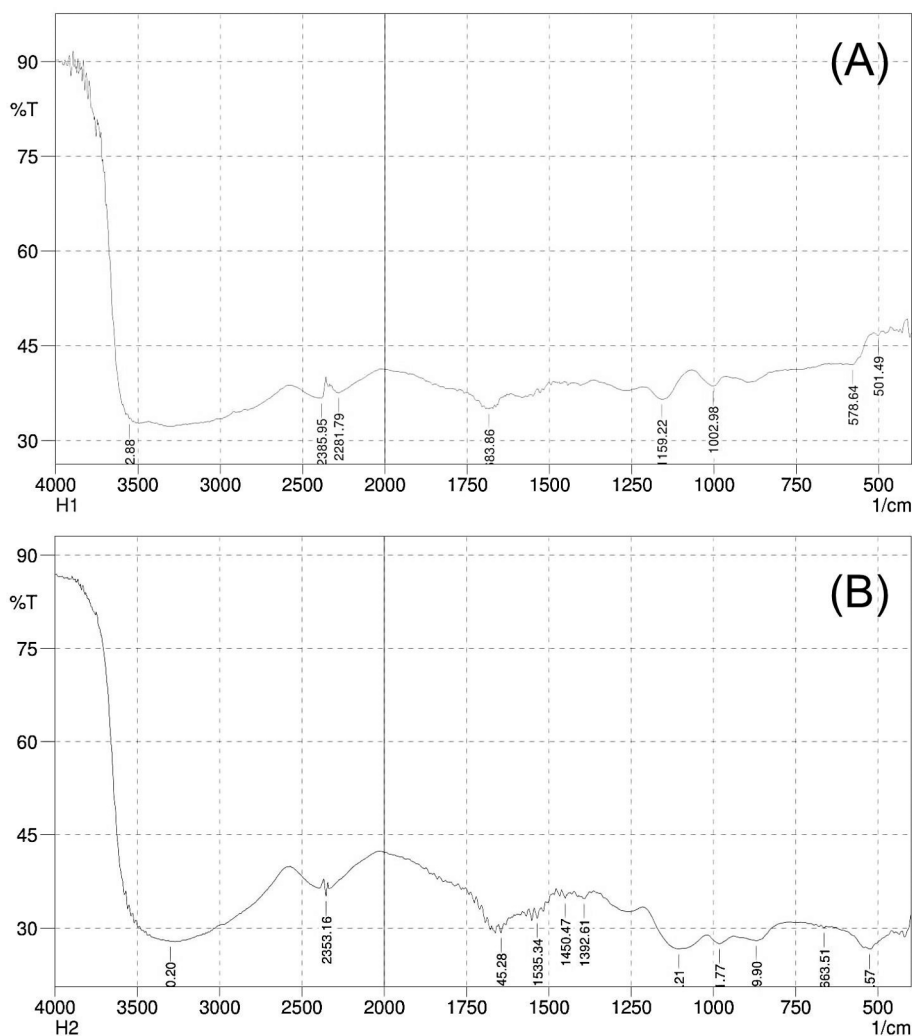

Fig. 8. FTIR transmittance curves for (A) Non-ultrasonic (NUS) hair hydrolysate and (B) Ultrasonic (US) hair hydrolysate.
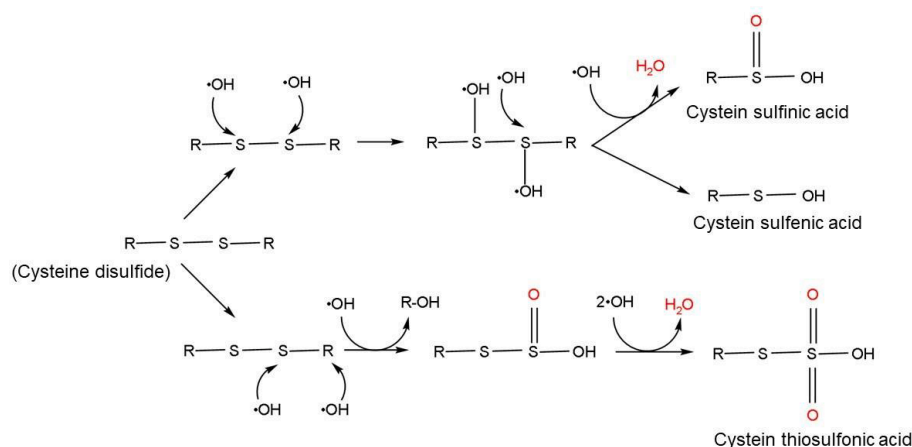
Fig. 9. Mechanism of hydroxyl radical attack on disulfide bonds in hair to form sulfinic acids and thiosulfonic acids; R = Cysteine (Cys). Readapted with permission from Holkar et al., 2016.

series of redox reactions. In the absence of disulfide bonds, electrons, and free radicals attack aromatic rings, peptide linkages and α-helical proteins deep inside hair fibers, causing the hair poly-peptides to depolymerize and lose strength [43,48].

The stronger peak around 1120 cm^{-1} for the US treated samples indicates the presence of cysteine S, S-dioxide and cysteine dioxide [48–50]. At the same time, deconvolution peak at around 1175 cm^{-1} seen in NUS samples, disappears in US samples, indicating the disappearance of sulfonate bonds to form sulfinic acids. All the changes observed with the increase in absorbance between 1200 and 900 cm^{-1} indicate the cleavage of disulfide bonds in the hair. The proposed mechanism for the cleavage of disulfide bonds includes the attack of hydroxyl radical to the sulfur linkages, agreeing with previously published mechanisms in wool and wool hydrolysates [3,28,48,49] (Fig. 9).

3.5. HPLC analysis

Three samples, viz. non-ultrasonic conventional, ultrasonically treated, and hydrodynamic cavitation treated, were analysed by HPLC for individual amino acid data. Ten amino acids, including alanine, cysteine, glycine, were analysed (Fig. 10). Amino acids were identified and quantified based on their retention times from standard procedures [51]. Several amino acids like leucine, lysine, isoleucine, arginine could not be identified due to the absence of retention peaks. Similar to the cleavage of cysteine bonds, poly-peptide bonds in hair fibre are ruptured on exposure to cavitation. Multiple amino acids are observed to be enhanced due to the breakage of poly-peptide bonds. Highest enhancement is seen in cysteine with hydrodynamic cavitation where the cysteine content increased from 0.001 μg L^{-1} to about 30 μg L^{-1}. The relatively higher number of disulfide bonds in cysteine make the enhancement possible [52,53]. Following cysteine, ornithine and taurine show the successively high improvement in individual amino acid content. Ornithine content increased from 0.3 μg L^{-1} to 230 μg L^{-1} in the cavitated sample, approximately 750 times increase in amino acid content. Out of all the amino acids present in hair, several nutritional amino acids were selected for quantification by HPLC. Enhancement in the content of nutritional amino acid when exposed to the US and HC treatment, as compared to conventional treatment, further strengthen the findings on keratinous substances like wool exposed to cavitating conditions [28]. Poly-peptide bonds in the hair make it highly stable in waste conditions, leading to low decomposition and degradability rates. Increase in the nutritional value of hair not only makes it a possible candidate for use in fertilizers but also makes it more degradable in soil.
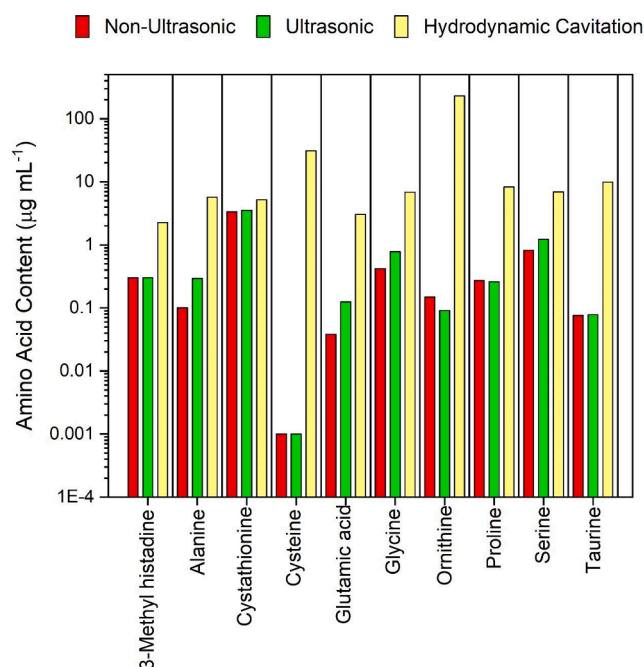

Fig. 10. Comparison of individual amino acid content obtained by HPLC after NUS treatment (red), US treatment (green), and HC treatment (yellow). Note that scale is in log-format and hence the increase in amino acid content is more substantial than seen in the representation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.6. Cost analysis

Techno-economic analysis is an integral part of the assessment of feasibility for novel technologies. Cost analysis for US and HC runs was performed for all powers and pressures (Table 2). The cost analysis was based on the energy consumption activities in the processes, i.e. direct power consumption of the ultrasonic transducer and power consumption by the pump for HC (Pump efficiency was approximately 60%). Since initial stirring is common to all processes, it was excluded from the analyses. Cost analysis has been calculated from Indian Rupees (₹), further converted to USD (1 ₹ = 0.013 USD) due to the localized average availability of cost at 10 ₹ KWh^{-1}.

Based on the cost per volume of solution treated, the cost for HC systems is approximately 20 times less than US systems. While the

Table 2
Costing analysis for ultrasonic (US) and hydrodynamic cavitation (HC) treatment based on net energy consumption.

Process	Power (W)/Pressure (bar)	Extent of viscosity reduction (%)	Time (minutes)	Energy per Liter (KWh/L)	Total Cost (USD m^{-3})	Cost per unit reduction in viscosity (USD m^{-3} viscosity reduction^{-1})
NUS	2.00 bar	–	15.00	7.5	1065	315
US	50.00 W	2.00	60.00	0.50	71.43	35.71
	100.00	3.40	60.00	1.00	142.86	42.02
	150.00	4.80	60.00	1.50	214.29	44.64
	200.00	9.60	60.00	2.00	285.71	29.76
HC	4.00 bar	4.50	30.00	0.005	0.68	0.15
	5.00	3.90	30.00	0.0073	1.05	0.266
	6.00	2.70	30.00	0.0103	1.483	0.55
	7.00	6.00	30.00	0.0138	1.98	0.33

viscosity reduction is higher in US systems, a 20-time higher cost is not feasible for the scale-up of the process. HC systems show potential promise due to its low cost while maintaining comparable results to US systems. For comparison purposes, the cost was also normalized to unit viscosity reduced. US systems still show substantially high costs in comparison to HC systems. These findings agree well with previously published reports comparing US and HC systems in terms of operating costs [43,54]. Even though the total operating cost of US system at 200 W is higher than that of 50–150 W (285 USD m^{-3} in comparison to 70 USD m^{-3}), when normalized to viscosity reduction 200 W operation performs better due to the substantially higher viscosity reduction (29 USD m^{-3} in comparison to 35 USD m^{-3}). In HC systems, even though 7 bar pressure yields a higher viscosity reduction as compared to 4 bar, the viscosity-normalized-cost for 4 bar pressure is much lower than 7 bar, indicating that 4 bar pressure could be optimum for a process scale-up. The findings present a strong trade-off between cost/energy consumption and relative viscosity reduction (associated depolymerization and chemical changes). The determination of optimum process parameters depends on the engineering constraints. Higher viscosity reduction and associated chemical changes also present higher operating costs. The low costs of HC treatment make it a potential candidate for scale-up of the process and development of pilot-scale methods.

In the conventional method in NUS systems using an autoclave, approximately 3 KW is consumed for operation at 120 °C and 5 bar, for 15 min. The energy consumption in the conventional method is higher than any cavitation process, while substantially higher than hydrodynamic cavitation.

3.7. Implications and potential applications

Hydrodynamic cavitation is an attractive alternative to conventional hydrolysis methods. A comparison to some previously published reports shows that HC is less energy intensive as well as consumes lesser time than conventional high temperature or high-pressure applications (Table 3). To our knowledge, there have been very few studies with recycling, valorization and re-use of human hair waste keratins. Alkaline hydrolysis yields much higher hydrolysis values as compared to its counterpart, acid-hydrolysis. Further, there have been studies on extraction and purification of amino-acids, and application of such hydrolysates in agricultural, biotechnological and food industries (Table 3).

Addition of orthophosphoric acid to hair hydrolysate make it's a potential candidate for use as organic amendment fertilizers. While the study of the application of the hair hydrolysate as fertilizers was out of scope for the current study, our previous study on keratinous hydrolysates has demonstrated successful application as an organic fertilizer, a potential alternative to synthetic fertilizers [28]. Enhancement of essential amino acids makes nitrogen easily available for plant uptake. Hair hydrolysates could provide essential micronutrients (C, N, and P), demonstrating an environmentally friendly alternative to synthetic fertilizers.

Cleavage of disulfide bonds in human hair allows for easier extraction of essential amino acids and proteins from hair [48]. Using physical processes like acoustic and hydrodynamic cavitation could avoid the use of harmful, toxic chemicals for oxidation and reduction of hair. Disulfide bonds in the hair make it stable in the soil while the cleavage of these bonds could help in increasing the biodegradability and decomposition

Table 3
Comparison of current study with previosuly published hydrolysis reports for certain keratinous wastes.

Keratinous waste	Hydrolysis	Quantity processed	Experimental details	Comments	Reference
Human Hair	Alkaline	1 g / 100 mL	4% KOH and 24 h initial dissolution for 24 h; US for 60 min at 200 W input power; HC at 4 or 7 bar for 30 min using venturi.	100% hydrolysis. Highly energy saving and cost saving in comparison to high temperature/pressure processes. Hydrodynamic cavitation is more cost efficient than ultrasonication.	Current study
Human Hair	Acid	50 g / 100 mL	20% HCl; Steam bath; 120 to 144 h.	Cystine is further separated and filtered. Dry filtered hydrolysate yield is about 5% of the initial material.	[15]
Human Hair	Alkaline	3.33 g / 100 mL	1–4% Sodium carbonate; steam bath for 16 h.	4–5% yield; Cystine separated and filtered. Energy intensive.	[17]
Human hair	Alkaline	12.5 g / 100 mL	0.5 N KOH and 0.05 N Ca(OH)2; 20 min at 120 °C.	pH adjusted using phosphoric acid. Microbial community in soil studied. Used as a fertilizer for hot pepper plants. Low yield.	[19]
Hog hair	Sub-critical water	5–15 g/L (protein based)	High temperature and high pressure reactor with heater; sealed and immersed in a heater for 360 min.	Individual amino acid enhancement studied. Parameters studied included temperature and time.	[20]
Wool	Alkaline	2 g / 100 mL	3% KOH and 24 h initial dissolution; 20 KHz and 40% amplitude for 30 min.	Increase in percentage hydrolysis with sonication. Application as fertilizer for growth of wheat.	[28]
Wool	Alkaline	1:3 fiber to liquid	KOH concentrations of 5–15% of fiber; superheated to 140–170 °C for 1 h.	Percentage hydrolysis unreported. Polypeptide degradation studied. Amino acids analysed. More energy intensive than cavitation.	[55]
Wool	Alkaline	2.5 to 20 g / 100 mL	0.15 M KOH and 0.05 M NaOH, 2 atm and 120 °C for 20 min.	Between 2 and 10% hydrolysis. Phosphoric acid neutralization and further demonstrated use as fertilizer.	[56]

of hair in the soil in comparison to untreated hair samples. Lesser compact structures of keratin sheets obtained from cavitation-based hydrolysis could improve the nutritional value of hydrolysates. Hair hydrolysates have potential application in enriching microbial communities and cultures. If extracted, essential amino acids obtained from hair hydrolysate could be used in food-grade poultry meals [50]. The use of protein-rich extracted products can reduce the generation of lower quality by-products in the manufacture of food-grade pet products [57] Higher digestibility needs to be investigated from an agricultural-research point of view. Hair hydrolysate could be an inexpensive and environmentally friendly substitute to amino-acid supplements.

4. Conclusions

Optimal conditions for initial hair hydrolysis for 100 mL of solution were 1 g waste human hair, 4 g KOH, 24 h of soaking. For ultrasonication, the optimal conditions were sonication time of 60 min with a power input of 200 W. For hydrodynamic cavitation; the optimal conditions were using 5 L of hair hydrolysate and an inlet pressure of 4 or 7 bars.

Cavitation results in rupture of disulfide linkages in proteins and mechanical effects lead to cleavage of several hydrogen bonds breaking the keratin sheet structure in hair. Breakdown of bonds leads to a decrease in viscosity of the solution. 10 and 6% reduction in viscosity is obtained at optimal conditions for ultrasonic and hydrodynamic cavitation treatment, respectively.

Cleavage of disulfide bonds leads to the formation of cysteine sulfinic acids and thiosulfonates, decreasing the strength of hair fiber. Cavitation leads to enhancement of amounts of individual amino acids by cleavage of protein in hair, increasing the nutritional value of hair hydrolysates.

Cost analysis reveals a trade-off between the chemical effects of cavitation and the cost of the process. Hydrodynamic cavitation with 4 bar pressure is the optimal condition for the lowest cost of process (0.68 USD m^{-3}). US runs cost approximately 20 times more than HC runs. Low process costs demonstrate a possibility of process scale-up. Application of hair hydrolysates to add nutritional value to fertilizers, soil and animal/poultry feed shows potential promise and needs to be investigated.

CRediT authorship contribution statement

Akash P. Bhat: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft, Writing - review & editing. **Chandrakant R. Holkar:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft, Writing - review & editing. **Ananda J. Jadhav:** Conceptualization, Formal analysis, Visualization, Writing - review & editing. **Dipak V. Pinjari:** Conceptualization, Funding acquisition, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2020.105368.

References

[1] A. Gupta, Human Hair "Waste"; and Its Utilization: Gaps and Possibilities, J. Waste Manag. 2014 (2014), e498018, https://doi.org/10.1155/2014/498018.

[2] K. Jagannathan, N. Panchanatham, AN OVERVIEW OF HUMAN HAIR BUSINESS IN CHENNAI, Int. J. Eng. Manag. Sci. 2 (2011) 199–204.

[3] M. Brebu, I. Spiridon, Thermal degradation of keratin waste, J. Anal. Appl. Pyrolysis. 91 (2011) 288–295, https://doi.org/10.1016/j.jaap.2011.03.003.

[4] C.R. Robbins, Chemical and Physical Behavior of Human Hair, 5th ed., Springer-Verlag, Berlin Heidelberg, 2012. //www.springer.com/gp/book/9783642256103 (accessed March 24, 2018).

[5] C.R. Robbins, Chemical Composition of Different Hair Types, in: Chem. Phys. Behav. Hum. Hair, Springer Berlin Heidelberg, 2012, pp. 105–176. https://doi.org/10.1007/978-3-642-25611-0_2.

[6] C. Popescu, H. Höcker, Hair—the most sophisticated biological composite material, Chem. Soc. Rev. 36 (2007) 1282–1291, https://doi.org/10.1039/B604537P.

[7] Characterization of a protease of a feather-degrading Microbacterium species - Thys - 2004 - Letters in Applied Microbiology - Wiley Online Library, (n.d.). http://onlinelibrary.wiley.com/doi/10.1111/j.1472-765X.2004.01558.x/full (accessed March 1, 2018).

[8] A.A. Onifade, N.A. Al-Sane, A.A. Al-Musallam, S. Al-Zarban, A review: Potentials for biotechnological applications of keratin-degrading microorganisms and their enzymes for nutritional improvement of feathers and other keratins as livestock feed resources, Bioresour. Technol. 66 (1998) 1–11, https://doi.org/10.1016/S0960-8524(98)00033-9.

[9] F.A. Banat, S. Al-Asheh, The Use of Human Hair Waste as a Phenol Biosorbent, Adsorpt. Sci. Technol. 19 (2001) 599–608, https://doi.org/10.1260/0263617011494420.

[10] K.A. Halhouli, N.A. Darwish, N.M. Al-Dhoon, Effects of pH and Inorganic Salts on the Adsorption of Phenol from Aqueous Systems on Activated Decolorizing Charcoal, Sep. Sci. Technol. 30 (1995) 3313–3324, https://doi.org/10.1080/01496399508013147.

[11] T.C. Tan, C.K. Chia, C.K. Teo, Uptake of metal ions by chemically treated human hair, Water Res. 19 (1985) 157–162, https://doi.org/10.1016/0043-1354(85)90193-9.

[12] V.D. Zheljazkov, J.L. Silva, M. Patel, J. Stojanovic, Y. Lu, T. Kim, T. Horgan, Human Hair as a Nutrient Source for Horticultural Crops, HortTechnology. 18 (2008) 592–596.

[13] V.D. Zheljazkov, Assessment of Wool Waste and Hair Waste as Soil Amendment and Nutrient Source, J. Environ. Qual. 34 (2005) 2310, https://doi.org/10.2134/jeq2004.0332.

[14] C.M. Williams, C.S. Richter, J.M. MacKenzie, J.C.H. Shih, Isolation, Identification, and Characterization of a Feather-Degrading Bacterium, Appl. Environ. Microbiol. 56 (1990) 1509–1515.

[15] R.A. Gortner, W.F. Hoffman, l-CYSTINE, Org. Synth. 5 (1925) 39, https://doi.org/10.15227/orgsyn.005.0039.

[16] K.R. Yadav, R.K. Sharma, M.P.S. Yadav, R.M. Kothari, Human hair waste: An environmental problem converted into an eco-friendly plant tonic, Fresenius Environ. Bull. 4 (1995) 491–496.

[17] W.F. Hoffman, Sulfur in Proteins Ii. the Effect of Mild Alkaline Hydrolysis Upon Hair, J. Biol. Chem. 65 (1925) 251–254.

[18] H.B. Merrill, Effect of Sulfides on the Alakline Hydrolysis of Skin and Hair, Ind. Eng. Chem. 17 (1925) 36–39, https://doi.org/10.1021/ie50181a016.

[19] S.O. Oh, A. Yun, D.H. Park, Effects of physicochemically hydrolyzed human hairs on the soil microbial community and growth of the hot pepper plant, Biotechnol. Bioprocess Eng. 16 (2011) 746–754, https://doi.org/10.1007/s12257-010-0467-3.

[20] M.B. Esteban, A.J. García, P. Ramos, M.C. Márquez, Sub-critical water hydrolysis of hog hair for amino acid production, Bioresour. Technol. 101 (2010) 2472–2476, https://doi.org/10.1016/j.biortech.2009.11.054.

[21] E.L. Pescatore, M.-S. Wu, The disulfide interchange reaction of hydrolyzed hair keratin and bis-dinitrophenyl cystine in concentrated hydrochloric-acid, J. Soc. Cosmet. Chem. 36 (1985) 363–371.

[22] E.A. Neppiras, Acoustic cavitation, Phys. Rep. 61 (1980) 159–251, https://doi.org/10.1016/0370-1573(80)90115-5.

[23] P.R. Gogate, V.S. Sutkar, A.B. Pandit, Sonochemical reactors: Important design and scale up considerations with a special emphasis on heterogeneous systems, Chem. Eng. J. 166 (2011) 1066–1082, https://doi.org/10.1016/j.cej.2010.11.069.

[24] M.P. Badve, P.R. Gogate, A.B. Pandit, L. Csoka, Hydrodynamic cavitation as a novel approach for delignification of wheat straw for paper manufacturing, Ultrason. Sonochem. 21 (2014) 162–168, https://doi.org/10.1016/j.ultsonch.2013.07.006.

[25] P.R. Gogate, A.B. Pandit, Hydrodynamic cavitation reactors: a state of the art review, Rev. Chem. Eng. 17 (2011) 1–85, https://doi.org/10.1515/REVCE.2001.17.1.1.

[26] P.R. Gogate, I.Z. Shirgaonkar, M. Sivakumar, P. Senthilkumar, N.P. Vichare, A. B. Pandit, Cavitation reactors: Efficiency assessment using a model reaction, AIChE J. 47 (2001) 2526–2538, https://doi.org/10.1002/aic.690471115.

[27] P.B. Baxi, A.B. Pandit, Using cavitation for delignification of wood, Bioresour. Technol. 110 (2012) 697–700, https://doi.org/10.1016/j.biortech.2012.01.042.

[28] C.R. Holkar, A.J. Jadhav, P.S. Bhavsar, S. Kannan, D.V. Pinjari, A.B. Pandit, Acoustic cavitation assisted alkaline hydrolysis of wool based keratins to produce organic amendment fertilizers, ACS Sustain. Chem. Eng. 4 (2016) 2789–2796, https://doi.org/10.1021/acssuschemeng.6b00298.

[29] C.R. Holkar, S.S. Jain, A.J. Jadhav, D.V. Pinjari, Valorization of keratin based waste, Process Saf. Environ. Prot. 115 (2018) 85–98, https://doi.org/10.1016/j.psep.2017.08.045.

[30] S. Koda, T. Kimura, T. Kondo, H. Mitome, A standard method to calibrate sonochemical efficiency of an individual reaction system, Ultrason. Sonochem. 10 (2003) 149–156, https://doi.org/10.1016/S1350-4177(03)00084-1.

[31] V.L. Gole, P.R. Gogate, Intensification of synthesis of biodiesel from non-edible oil using sequential combination of microwave and ultrasound, Fuel Process. Technol. 106 (2013) 62–69, https://doi.org/10.1016/j.fuproc.2012.06.021.

[32] S. Raut-Jadhav, V.K. Saharan, D. Pinjari, S. Sonawane, D. Saini, A. Pandit, Synergetic effect of combination of AOP's (hydrodynamic cavitation and H2O2) on the degradation of neonicotinoid class of insecticide, J. Hazard. Mater. 261 (2013) 139–147, https://doi.org/10.1016/j.jhazmat.2013.07.012.

[33] M.M. Gore, V.K. Saharan, D.V. Pinjari, P.V. Chavan, A.B. Pandit, Degradation of reactive orange 4 dye using hydrodynamic cavitation based hybrid techniques, Ultrason. Sonochem. 21 (2014) 1075–1082, https://doi.org/10.1016/j.ultsonch.2013.11.015.

[34] P.R. Gogate, A.L. Prajapat, Depolymerization using sonochemical reactors: A critical review, Ultrason. Sonochem. 27 (2015) 480–494, https://doi.org/10.1016/j.ultsonch.2015.06.019.

[35] A.L. Prajapat, P.R. Gogate, Intensification of depolymerization of aqueous guar gum using hydrodynamic cavitation, Chem. Eng. Process. Process Intensif. 93 (2015) 1–9, https://doi.org/10.1016/j.cep.2015.04.002.

[36] A.L. Prajapat, P.R. Gogate, Depolymerization of guar gum solution using different approaches based on ultrasound and microwave irradiations, Chem. Eng. Process. Process Intensif. 88 (2015) 1–9, https://doi.org/10.1016/j.cep.2014.11.018.

[37] A.L. Prajapat, P.R. Gogate, Intensified depolymerization of aqueous polyacrylamide solution using combined processes based on hydrodynamic cavitation, ozone, ultraviolet light and hydrogen peroxide, Ultrason. Sonochem. 31 (2016) 371–382, https://doi.org/10.1016/j.ultsonch.2016.01.021.

[38] A.L. Prajapat, P.B. Subhedar, P.R. Gogate, Ultrasound assisted enzymatic depolymerization of aqueous guar gum solution, Ultrason. Sonochem. 29 (2016) 84–92, https://doi.org/10.1016/j.ultsonch.2015.09.009.

[39] A.L. Prajapat, P.R. Gogate, Depolymerization of carboxymethyl cellulose using hydrodynamic cavitation combined with ultraviolet irradiation and potassium persulfate, Ultrason. Sonochem. 51 (2019) 258–263, https://doi.org/10.1016/j.ultsonch.2018.10.009.

[40] A.L. Prajapat, P.R. Gogate, Intensification of depolymerization of polyacrylic acid solution using different approaches based on ultrasound and solar irradiation with intensification studies, Ultrason. Sonochem. 32 (2016) 290–299, https://doi.org/10.1016/j.ultsonch.2016.03.022.

[41] Y.T. Shah, A.B. Pandit, V.S. Moholkar, Cavitation Reaction Engineering, Springer Science & Business Media, 2012.

[42] P. Senthil Kumar, M. Siva Kumar, A.B. Pandit, Experimental quantification of chemical effects of hydrodynamic cavitation, Chem. Eng. Sci. 55 (2000) 1633–1639, https://doi.org/10.1016/S0009-2509(99)00435-2.

[43] V.V. Patil, P.R. Gogate, A.P. Bhat, P.K. Ghosh, Treatment of laundry wastewater containing residual surfactants using combined approaches based on ozone, catalyst and cavitation, Sep. Purif. Technol. 239 (2020), 116594, https://doi.org/10.1016/j.seppur.2020.116594.

[44] N. Eslahi, F. Dadashian, N.H. Nejad, An investigation on keratin extraction from wool and feather waste by enzymatic hydrolysis, Prep. Biochem. Biotechnol. 43 (2013) 624–648, https://doi.org/10.1080/10826068.2013.763826.

[45] S. Hilterhaus-Bong, H. Zahn, Contributions to the chemistry of human hair. 1. Analyses of cystine, cysteine and cystine oxides in untreated human hair, Int. J. Cosmet. Sci. 9 (1987) 101–110, https://doi.org/10.1111/j.1467-2494.1987.tb00467.x.

[46] T.M. Florence, Degradation of protein disulphide bonds in dilute alkali, Biochem. J. 189 (1980) 507–520.

[47] J. Strassburger, M.M. Breuer, Quantitative Fourier transform infrared spectroscopy of oxidized hair, J Soc Cosmet Chem. 36 (1985) 61–74.

[48] K.R. Millington, J.S. Church, The photodegradation of wool keratin II. Proposed mechanisms involving cystine, J. Photochem. Photobiol. B. 39 (1997) 204–212, https://doi.org/10.1016/S1011-1344(96)00020-6.

[49] M. Joy, D. Lewis, The use of Fourier transform infra-red spectroscopy in the study of the surface chemistry of hair fibres, Int. J. Cosmet. Sci. 13 (1991) 249–261.

[50] J. Kong, S. Yu, Fourier transform infrared spectroscopic analysis of protein secondary structures, Acta Biochim. Biophys. Sin. 39 (2007) 549–559.

[51] V. Davankov, A. Bochkov, A. Kurganov, P. Roumeliotis, K. Unger, Separation of unmodified α-amino acid enantiomers by reverse phase HPLC, Chromatographia. 13 (1980) 677–685.

[52] M.A. Manuszak, E.T. Borish, R.R. Wickett, The kinetics of disulfide bond reduction in hair by ammonium thioglycolate and dithiodiglycolic acid, J. Soc. Cosmet. Chem. 47 (1996) 49.

[53] L.J. Wolfram, D.L. Underwood, The Equilibrium Between the Disulfide Linkage in Hair Keratin and Sulfite or Mercaptan, Text. Res. J. 36 (1966) 947–953, https://doi.org/10.1177/004051756603601102.

[54] M. Gągol, A. Przyjazny, G. Boczkaj, Wastewater treatment by means of advanced oxidation processes based on cavitation – A review, Chem. Eng. J. 338 (2018) 599–627, https://doi.org/10.1016/j.cej.2018.01.049.

[55] P. Bhavsar, M. Zoccola, A. Patrucco, A. Montarsolo, G. Rovero, C. Tonin, Comparative study on the effects of superheated water and high temperature alkaline hydrolysis on wool keratin, Text. Res. J. 87 (2016) 1696–1705, https://doi.org/10.1177/0040517516658512.

[56] A. Gousterova, M. Nustorova, I. Goshev, P. Christov, D. Braikova, K. Tishinov, T. Haertlé, P. Nedkov, Alkaline Hydrolysate of Waste Sheep Wool Aimed as Fertilizer, Biotechnol. Biotechnol. Equip. 17 (2003) 140–145, https://doi.org/10.1080/13102818.2003.10817072.

[57] W.A. Dozier, N.M. Dale, C.R. Dove, Nutrient Compostion of Feed-Grade and Pet-Food-Grade Poultry By-Product Meal, J. Appl. Poult. Res. 12 (2003) 526–530, https://doi.org/10.1093/japr/12.4.526.