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Study of a complex protein foaming agent from disintegrated brewery sludge supernatant

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ABSTRACT

In this study, disintegrated brewery sludge supernatant was used to make complex protein foaming agent (CPFA), which was applied in cellular concrete. The influences of CFPA proportion, additives and temperature were investigated. Foam property was measured by the Ross–Miles method and the optimal foaming condition for CPFA was found to be when the complex proportion was 1:60 to disintegrated supernatant and the temperature 40°C. The relationship of foam property (Y) and dosage of additives (gum arabic X_1, sucrose X_2 and zinc sulfate X_3) was a polynomial. CFPA was used to produce cellular concrete and its performance contrasted with the concrete made using an animal protein foaming agent. The density of CPFA concrete was very similar to that of animal protein concrete, but the former's compressive strength, sorptivity and pore structure found to be superior. Applied disintegrated brewery sludge supernatant as a foaming agent, not only can produce economic benefit, but also solve a growing environmental problem. This study provided a new idea in the resource utilization of brewery sludge.

Keywords: Disintegrated supernatant; Complex protein; Foaming agent; Foam property; Cellular concrete

1. Introduction

In recent years, with the rapid development of the brewing industry in China [1,2], it has become one of the world's largest beer producers. The brewing industry produces large quantities of wastewater with a high concentration of degradable organic pollutants and excess sludge [3,4]. As market demand leads to rising beer production, increased sludge production grows apace [5]. Brewery sludge generated during brewery wastewater treatment must be treated to reduce its volume and environmental risk. Many traditional means have been reported regarding means for successfully disposing of sludge, including composting, landfill and incineration [6]. Traditional disposal routes have given rise to the environmental and regulatory problems, because of the organic components in sludge and the limited amount of available land for disposal. Thus, the development of effective waste sludge treatment technology is critical for sustainable growth of the brewery industry.

Disintegration of sludge which can break bacterial cell walls increases the biodegradability of sludge and favors reduced sludge production. Sludge disintegration consists of a group of processes, due to the influence of mechanical effect, chemical action and heat [7,8]. The operation hydrolyzes the macromolecules, such as extracellular polymeric substances, and disrupts cells to release intracellular organics into the supernatant [9,10]. Excess sludge consists of valuable organic substances, such as nucleic acids, enzymes, proteins and polysaccharides [11,12]. Protein is abundant in sludge flocs and microbial cells and represents a major fraction of sludge organic matter [13–15]. Beer production uses yeast for fermenting the raw material grain [16]. As quantities of waste

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yeast are released with wastewater and sludge [17], the protein content of brewery sludge is higher than municipal sewage sludge. To usefully employ brewery sludge protein would be beneficial to in terms of resource utilization and serve to create economic, environmental and social profits. Previously, this research carried out in this research group has shown that the mechanical disruption method combined with chemical treatment was useful for the release of protein in brewery sludge. The highest rate of protein release into disintegrated supernatant is ~65%, with the content up to 1,200 mg/L.

The materials components of cellular concrete include cement, water, aggregate and air void where foam that has been mixed into mortar or concrete [18]. As cellular concrete is lighter and possesses good insulation properties, the application of it in building is popular [19,20]. Protein is widely used in cellular concrete as the foaming agent, as it possesses good foam properties [21]. Protein foaming agent contains animal and plant protein foaming agent [22]. Holes in the cellular concrete are generated by employing either a prefoaming method or mixed foaming method [23]. Protein foaming agents are widely used around the world, but as a resource, it is limited and its price is high. Protein in the brewery sludge offers an alternative source of foaming protein agent and, if the brewery sludge was used as a source of foaming agent, it would provide the economic and environmental benefits as well as ameliorate part of the sludge disposal challenge.

However, to the best of our knowledge, the potential of the complex protein foaming agent (CPFA) from brewery sludge has not been assessed in date in any previous literature. The processes of protein purification and concentration are complicated, and there are probably protein loses during these processes [24]. In this study, disintegrated brewery sludge supernatant was combined directly with animal protein foaming agent (APFA), the foam property of CPFA discussed and the effects of using CPFA incorporating into a cellular concrete were examined.

2. Materials and methods

2.1. Sludge sampling

In this study, sludge sampling came from the wastewater treatment station is one of the largest breweries in Nanjing China. The annual production of sludge in this station was ~540,000 tons. The samples were stored before experimentation for not more than 48 h at 4°C in a refrigerator. The characteristics of the sampling sludge are shown in Table 1.

2.2. Sludge disintegration

An alkali-ball milling method was used to disintegrate brewery sludge, at pH = 12, milling beads dosage of 300 g/L and stirring intensity 500 rpm (Tables S1 and S2 of supplementary material). The concentration of released protein into the liquid was determined by centrifuging the sludge at 6,000 rpm for 30 min and, then, vacuum filtration through a membrane cellulose acetate filter (SCBB-206, ANPEL Laboratory Technologies Inc., Shanghai) with 0.45 μm pore diameter. Protein concentrations were measured by the Lowry method [9]. The properties of disintegration supernatant are shown in Table 2.

2.3. Foam property of complex protein foaming agent

The optimal foaming conditions for CPFA were investigated by assessing the influence of CPFA ratios (APFA:disintegrated supernatant), various concentrations of additives and temperature of circular water. The different ratios of APFA to disintegrated supernatant were 1:20, 1:30, 1:40, 1:50, 1:60, 1:70 and 1:80 (by volume). The various dosages of additives (zinc sulfate, sucrose and gum arabic) were 1.25, 2.5, 3.75, 5, 6.25, 7.5, 8.75, 10, 11.25 and 12.5 g/L CPFA. The circulating water temperature was set at 10°C, 20°C, 30°C, 40°C, 50°C and 60°C. The properties of foaming agents were detected using a Ross-Miles foam apparatus (Wilmad-LabGlass, Vineland, NJ, USA; Fig. 1) [25,26]. The initial foam height characterized an agent's foaming property and the foam height after 5 min was contrasted with the initial height to characterize the foam stability.

Table 1
Characteristics of brewery waste sludge

Item	Average	Deviation
pH	7.7	0.2
MLSS (g/L)	19.8	0.3
MLVSS (g/L)	8.2	0.9
SCOD (mg/L)	136.5	6.5
TN (mg/L)	81.2	9.6
TP (mg/L)	41.7	0.7
Soluble protein (mg/L)	109.7	5.2
Rate of water content (%)	98.0	0.1

SCOD – dissolved chemical oxygen demand; and TN – total nitrogen.

Table 2
Properties of disintegrated supernatant

Item	pH	COD (mg/L)	TN (mg/L)	TP (mg/L)	Soluble protein (mg/L)
	8.9	1,092	406	250.2	767.9

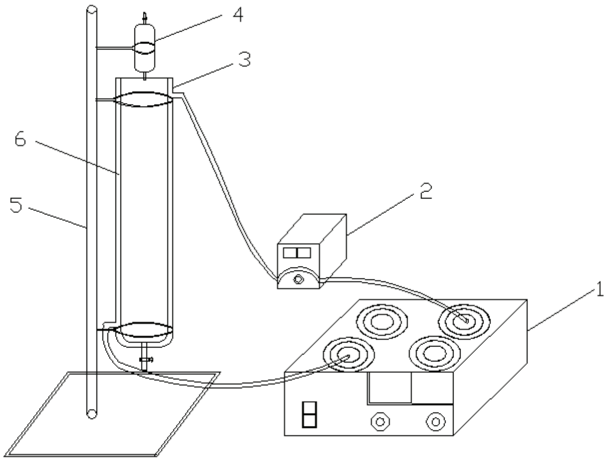

Fig. 1. Schematic diagram of the foaming property text apparatus. 1, Digital thermostat bath pot; 2, constant peristaltic pump; 3, graduated tube; 4, dropping tube; 5, iron bracket and 6, insulation layer.

2.4. Preparation and curing of cellular concrete

The preparation of cellular concrete was in accordance with CSA A23.2-2C-09 [27] and CSA A23.2-3C-09 [28]. The general formula used here included cement, natural sand and crushed stone. APFA was supplied by a company in Anhui, China. The maximum APFA dilution was 40-fold, at which point the initial foam height was 204 mm and 5 min height at 185 mm. The foam was prepared by rapid stirring method [23]. Concrete cylinders (100 × 200 mm, diameter and height, respectively) and concrete cubes (100 × 100 × 100 mm) were cast for testing the resulting properties. Specimens were demolded after 24 h and then naturally cured for 28 d.

2.5. Analytical methods

The majority of brewery waste sludge characteristics were analyzed by Standard Methods [29], and the closed reflux method employed for chemical oxygen demand (COD) [30]. For the analysis of total phosphorous (TP), samples were treated using the alkaline persulfate digestion method [31]. Mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS) and water content were measured by gravimetric methods.

The density of concrete was the ratio of the weight to volume, measured by cubes dried in an electric thermostatic drying oven until the weight was constant [32]. The compressive strength of concrete specimens was measured at ages 7 and 28 d, in accordance to ASTM C39-05 [33]. The sorptivity was measured in accordance with ASTM C1585-04 [34]. The microscopic images of cellular concrete pores were examined by environmental scanning electron microscopy (Quanta 200, FEI Company, Hillsboro, USA).

3. Results and discussion

3.1. Effects of CPFA proportion on foam property

The foaming property of CPFA decreased with its increased proportion to disintegrated supernatant (Fig. 2). When the proportion was 1:60, the CPFA foaming property was equivalent to the animal protein at the maximum dilution (initial foam height and 5 min the height at 204 and 185 mm, respectively), but foam stability was slightly decreased. When the APFA:disintegrated supernatant was 1:70 and 1:80, the foaming property was too low. The addition of disintegrated supernatant improved the dilution ratio of APFA and indicated that the amount of APFA used in practical engineering could be reduced. Protein in the supernatant increased the protein concentration of CPFA, this improving its foam property. Therefore, the CPFA was feasible.

3.2. Effects of additives on CPFA foam property

3.2.1. Effects of the inorganic additive zinc sulfate

Zinc sulfate addition improved the agent's foaming properties, and foam stability also improved with increased dosage (Fig. 3). Zn^{2+} and SO_4^{2-} in solution formed an ion environment that was conducive to generating foam and improving foam stability. Amphoteric protein molecules were adsorbed on the foam surface after foam formation, which made the liquid film surface electrically charged. The addition of zinc sulfate, with divalent cations and anions, stabilized the electrostatic repulsions, preventing liquid film drainage and improving the foam property (Fig. 3). During the experiment, high zinc sulfate dosage was found to produce flocs (Fig. 4). This indicated that the protein was salted-out with excess zinc sulfate addition, which reduced the protein content in the foaming agent and destroyed the foam property.

3.2.2. Effects of the small organic molecules additive sucrose

Sucrose improved the foaming property of CPFA but decreased the foam stability (Fig. 5), which indicated that sucrose had adverse effects on protein activity. However, when the dosage was >7.5 g/L, the foam stability performance improved significantly, which was because sucrose increased the viscosity of the liquid phase and improved the viscosity of the foam film as well, which was beneficial for enhancing its flexibility and toughness.

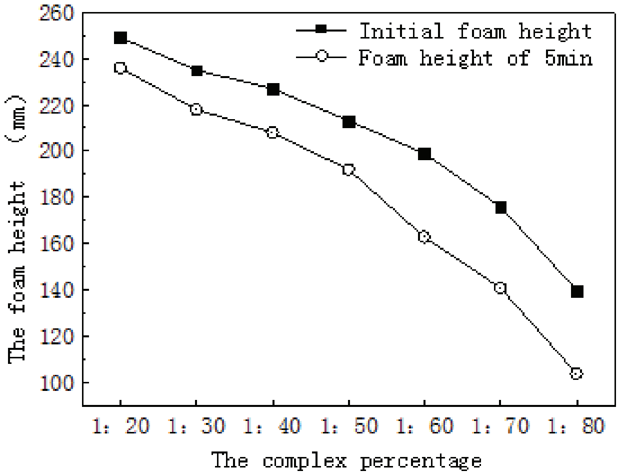

Fig. 2. Effects of APFA: disintegrated supernatant on CPFA foam property.

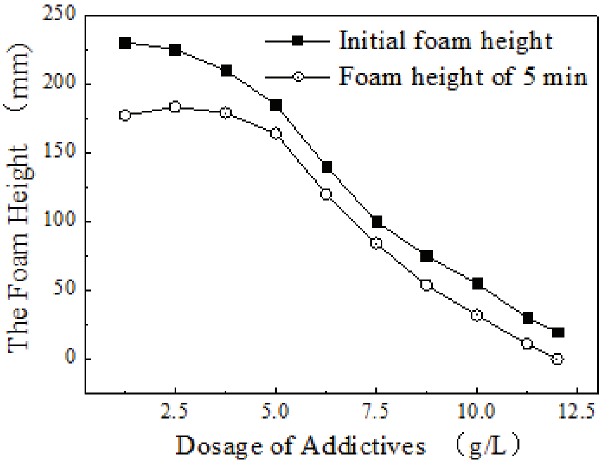

Fig. 3. Effects of zinc sulfate on CPFA foam property.

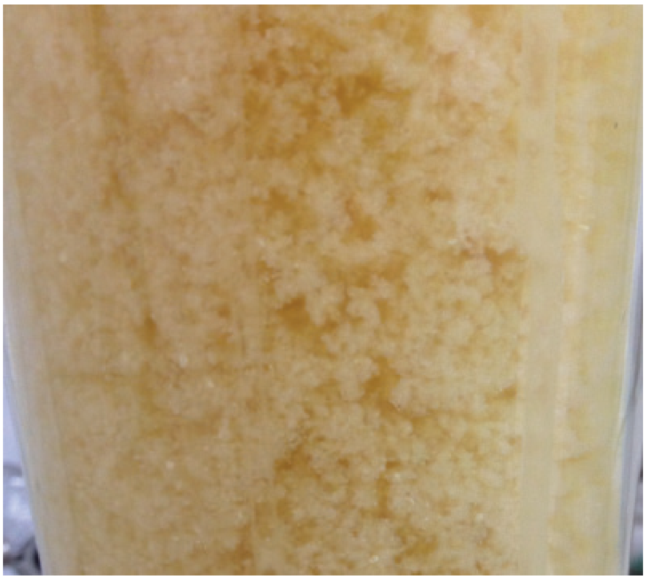

Fig. 4. Salted-out of CPFA, foaming floc.

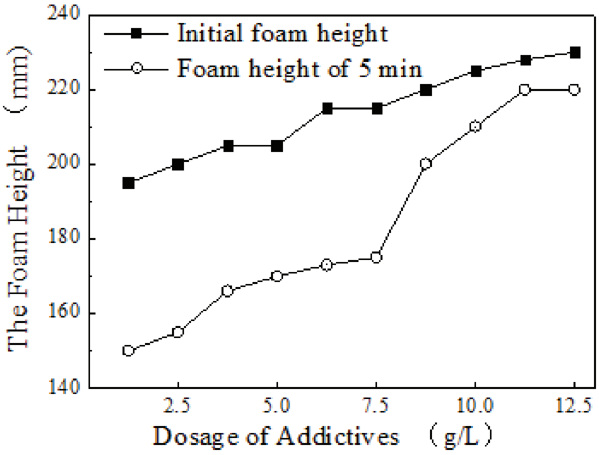

Fig. 5. Effects of sucrose on CPFA foam property.

3.2.3. Effect of organic macromolecules additive gum arabic

When the dosage of gum arabic was <3.75 g/L, the foam property was enhanced as the dosage increased from low to this limit (Fig. 6). However, when the dosage was >3.75 g/L, increased dosage has adverse effects. Gum arabic is commonly used as an industrial thickener, which increases the viscosity of the foaming agents and decreases permeability, thus improving foam quality and stability. Here, however, gum arabic dosing made the foaming agent too thick and increased viscous resistance, which was not conducive to foam production.

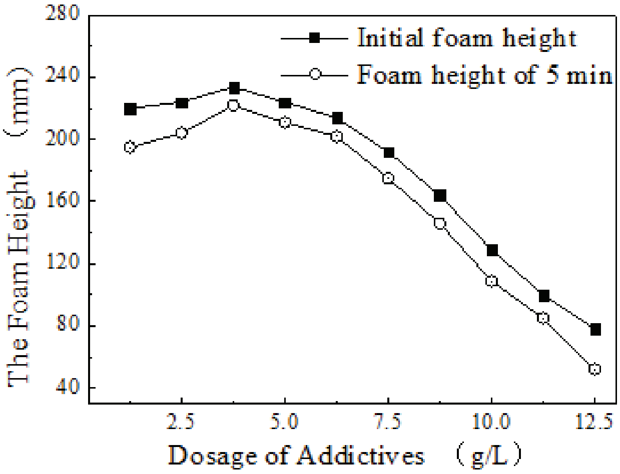

Fig. 6. Effect of gum arabic on CPFA foam property.

3.2.4. Effect of combined additives

As each additive exhibited its own advantages and disadvantages, the goal of this study was to investigate the comprehensive effect of the three additives in decreasing the side effects of each individual additive. Thus, the three additives, zinc sulfate, sucrose and gum arabic, were combined to examine their synthetic impact on the foam property. The regression orthogonal experiment was designed to investigate the influences and to elaborate dosage relationships. The dosages of gum arabic X_1, sucrose X_2, and zinc sulfate X_3 were 2.5–5.0, 8.75–11.25 and 1.25–3.75 g/L, respectively. The composite index (F_q) was introduced to evaluate the foam property and calculated as:

$$F_q = \int_{t_0}^{t_5} h(t)\mathrm{d}t \tag{1}$$

where t_0 and t_5 represent the initial time and after 5 min, respectively, and $h(t)$ the foam height at time t. Supposing a bubble burst rate constant, then Eq. (1) simplified to:

$$F_q = \frac{5}{2}(h_0 + h_5) \tag{2}$$

where h_0 and h_5 represent the height at the initial time and after 5 min, respectively.

Using Y to represent the foam composite index, the factor levels were encoded (Table 3) and the test results shown in Table 4.

The regression equation was established by analyzing the data and calculating the regression coefficients, which were Y = 1,174.318 + 33.438Z_1 + 62.813Z_3 – 27.813Z_3. According to the absolute value of the partial regression coefficient, the order of additives impacts on the foam property was sucrose > gum arabic > zinc sulfate. When the dosage of sucrose was 11.25 g/L, gum arabic 5.0 g/L and zinc sulfate 1.25 g/L, the foam property was optimal.

After variance analysis, the significant of three factors and the regression equation was high, but the lack of fit test of regression equation was not significant. Therefore, the regression equation established by ternary regression orthogonal experiment fit with the actual situation appropriately. Substituted the coding formula back into the regression equation was $Y = 627.126 + 26.75X_1 + 50.25X_2 – 22.25X_3$.

3.3. Effect of temperature on CPFA foam property

The influence of temperature on the foam property of CPFA was relatively clear, while the foam stability was not significantly affected (Fig. 7). As temperature increased, the gas diffusion rate rose which gave rise to promoting gas–liquid contacts that were conducive to foam formation. Higher temperature caused the gases inside the bubbles to diffuse outward and eventually led to bubble collapse. At the same time, gum arabic increased bubble toughness, enhancing resistance to gas diffusion, thus suppressing bubble failure. As the rate of bubble production was faster than bubble bursts, the amount of foam increased. When the temperature was higher than 40°C, the foam property changed little and, thus, 40°C was chosen as the optimal temperature.

3.4. Properties of cellular concrete

3.4.1. Performance of cellular concrete

The densities of CPFA and APFA cellular concretes were not significantly different (Table 5). However, the compressive strength and sorptivity of CPFA cellular concrete was slightly higher than the other. Observation of the pore distribution in concrete surfaces showed that pores of CPFA cellular concrete were smaller and more evenly distributed than those in APFA concrete (Fig. 8).

Compressive strength is affected by density, the water–cement ratio and foam distribution [35]. These two kinds of cellular concrete differed little in terms of density and water–cement ratio after the production process, such that their compressive strengths were mainly affected by the foam's pore distribution. The pore distribution in CPFA cellular concrete was even more closely than APFA cellular concrete, which suggested that the foam mixed into mud more uniformly (Fig. 8). This was the reason why the compressive strength of CPFA cellular concrete was slightly higher than in APFA concrete. Sorptivity is related to the porosity of cellular concrete and the higher the porosity, the stronger capillary water absorption effect, which results in the higher sorptivity. Thus, the CPFA cellular concrete possessed a higher sorptivity than the other concrete.

3.4.2. The pore structure of cellular concrete

The shapes of pores in CPFA cellular concrete were approximately spherical and uniform size distribution

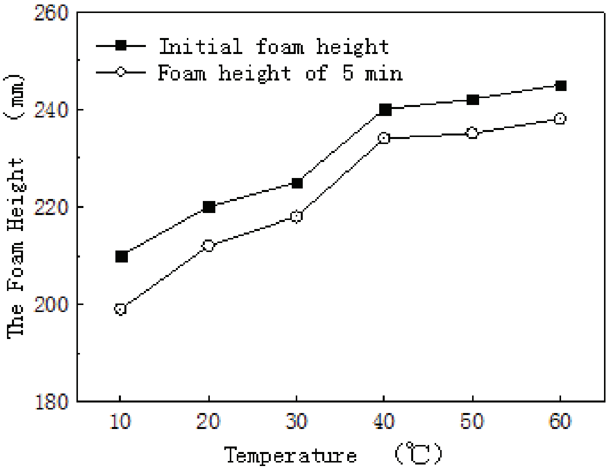

Fig. 7. Effects of temperature on CPFA foam property.

Table 3
Coding list of factors levels of orthogonal regression experiment

Z_j	Gum arabic X_1 (g/L)	Sucrose X_2 (g/L)	Zinc sulfate X_3 (g/L)
Up level (1)	5.0	11.25	3.75
Down level (–1)	2.5	8.75	1.25
Zero level (0)	3.75	10	2.5
Change pitch Δ_j	1.25	1.25	1.25

Table 5
Characteristics of cellular concrete

Cellular concrete type	Density (kg/m^3)	Compressive strength (MPa)	Sorptivity (%)
APFA	1.10	1.22	25.67
CPFA	1.08	1.83	29.34

Table 4
Design and result of orthogonal regression experiment

No.	Z_1	Z_2	Z_3	X_1 (g/L)	X_2 (g/L)	X_3 (g/L)	h_0 (mm)	h_5 (mm)	Y
1	1	1	1	5.0	11.25	3.75	253	236	1,222.5
2	1	1	–1	5.0	11.25	1.25	265	253	1,295.0
3	1	–1	1	5.0	8.75	3.75	237	215	1,130.0
4	1	–1	–1	5.0	8.75	1.25	242	227	1,172.5
5	–1	1	1	2.5	11.25	3.75	248	223	1,177.5
6	–1	1	–1	2.5	11.25	1.25	259	238	1,242.5
7	–1	–1	1	2.5	8.75	3.75	222	196	1,045.0
8	–1	–1	–1	2.5	8.75	1.25	228	207	1,087.5
9	0	0	0	3.75	10	2.5	245	230	1,187.5
10	0	0	0	3.75	10	2.5	240	227	1,167.5
11	0	0	0	3.75	10	2.5	243	233	1,190.0

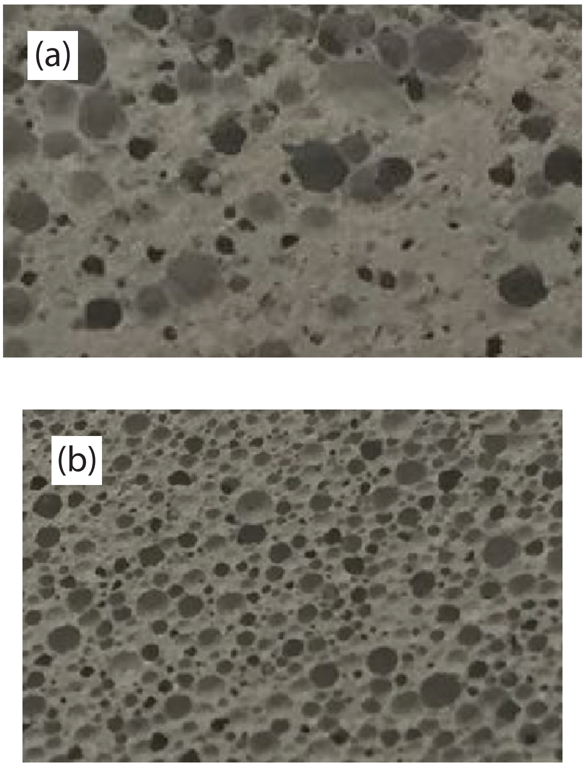

Fig. 8. Pores distribution in cellular concrete. Cellular concrete made from (a) APFA and (b) CPFA.

(Fig. 9). Also, the pores were independent and did not connect with each other. The pore shapes in APFA cellular concrete were more complex, including a number of oval and an irregular shape, and contained many interbubble channels formed by bubble breakage. The microscopic structures in these cellular concrete explained the differences in their macroscopic properties.

4. Conclusions

This study examined protein from disintegrated brewery sludge supernatant as a new resource of foaming agent and investigated the impact of mixtures with APFA (CPFA), additives and temperature on foam property. When the APFA:disintegrated supernatant was 1:60 and the temperature was 40°C, the foam property was optimal. The side effects of single additives were reduced by investigating additive combinations using the regression orthogonal experiment designed to assess the comprehensive impact of three additives. From this analysis, the optimal concentrations of the combined additives were determined. When CPFA was used to form cellular concrete, its performance was better than a similar APFA concrete. This technology can facilitate the use of protein-rich CPFA for economic benefit as well as reduce the difficulty of brewery sludge disposal.

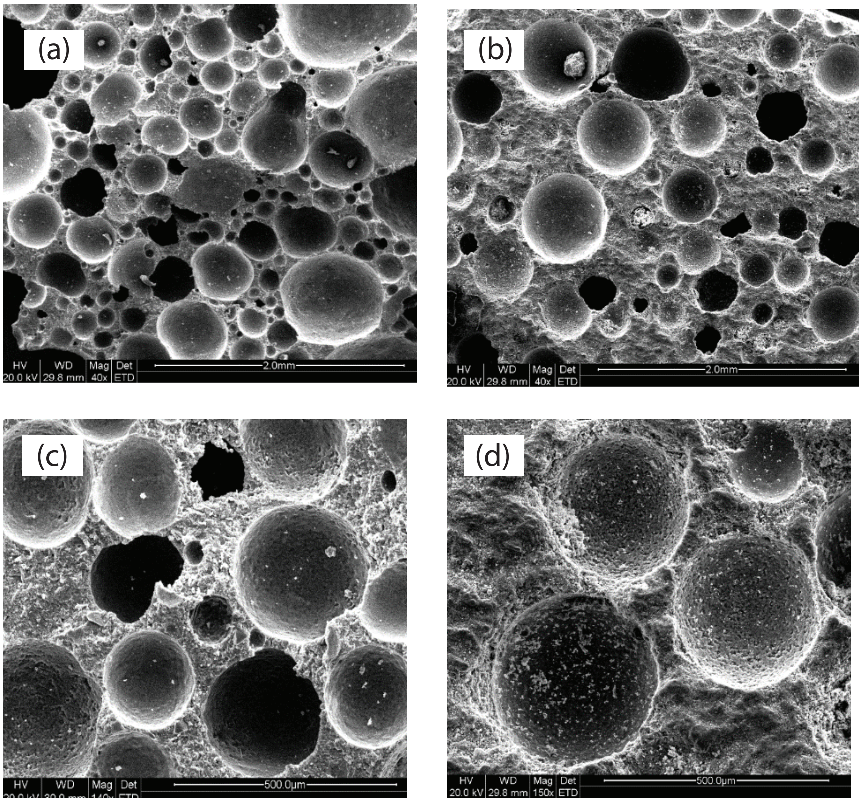

Fig. 9. Environmental scanning electron microscope image of cellular concrete pores. APFA and CPFA cellular concrete magnified of 40×, (a) and (b), respectively, with (c) and (d) at 150×, respectively.

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Supplementary material

Table S1
Orthogonal experimental design and results of alkali-ball mill measure to break sludge

Factors	pH	The dosage of milling ball (g)	Stirring intensity (*r*/min)	Protein extraction ratio (mg/g)
1	10	40	300	26.71
2	10	45	400	32.69
3	10	50	500	34.96
4	11	40	400	39.51
5	11	45	500	42.19
6	11	50	300	36.40
7	12	40	500	65.52
8	12	45	300	40.52
9	12	50	400	59.91
Mean value 1	31.453	43.913	34.543	
Mean value 2	39.367	38.467	44.037	
Mean value 3	55.317	43.757	47.557	
Range	23.864	5.446	13.014	
Factors exerted varying	I	III	II	
Optimal levels	12	40	500	

Protein extraction ratio: the quantity of protein in disintegrated supernatant (mg)/the weight of dry sludge.

Table S2
Orthogonal experimental variance analysis of alkali-ball mill measure to break sludge

Sources of variation	Deviation sum of squares	Degrees of freedom	Mean square	*F*	$\lambda_{0.05}$
pH	886.482	2	443.241	13.088	19.000
The dosage of milling ball	57.675	2	28.838	0.852	19.000
Stirring intensity	271.861	2	135.931	4.014	19.000
SUM	1,216.018	8			

SUM: the summary of the three factors' deviation sum of squares and degrees of freedom, respectively.