

# Potential Evaluation of Biogas Production and Upgrading Through Algae

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**Abstract**— Algae are known to be a potential feedstock in the production of biogas. Many studies has been focused on microalgal species, macroalgae are also suitable as a source of rich protein, carbohydrate and lipids. In this study, a locally abundant (Chiang Mai Province, Thailand) and naturally grown filamentous algae, *Spirogyra ellipsospora* has been harvested from a slow running freshwater stream; subsequently biomass was dried with solar dryer, and the materials were pulverized for chemical composition analysis. The feasibility of utilizing macroalgae biomass as a monosubstrate for the biogas production was investigated. Results showed that the highest methane yield was reached 65 % without any pretreatment process. This study suggested that it is possible to achieve stable operation using *S. ellipsospora*, as a substrate for biogas production in pilot or large scale biogas plant. Therefore, *S. ellipsospora* as energy crop can be an alternative energy resource.

**Index Terms**— Algae, Freshwater stream, *Spirogyra ellipsospora*, *Chlorella vulgaris*, Biogas.

## I. INTRODUCTION

Due to the energy crisis, renewable energy becomes a popular issue in this world today and there are several alternatives such as bioenergy, solar, wind, tide, geothermal, etc. Bioenergy is a type of renewable energy made from biological sources including algae, trees, or waste from agriculture, wood processing, food materials, and municipalities. For bioenergy, algae are the third generation biofuel [1]. It provides an excellent biomass as a renewable energy source, so called “bioenergy”, and turn algae as the most efficient bio-component [2]. Recently, macroalgae have recently received considerable attentions as a substrate for biofuels production, since they have higher growth rates compared to the plants. The average photosynthetic efficiency of aquatic biomass is 6–8%, which is much higher than that of terrestrial biomass (1.8–2.2%).

Macroalgae are fast growing marine and freshwater plants that can grow to considerable size (up to 60 m in length). Annual primary production rates (grams cm<sup>-2</sup> yr<sup>-1</sup>) are higher for the major marine macroalgae than for most terrestrial biomass [3]. Macroalgae can be subdivided into the blue algae (Cyanophyta), green algae (Chlorophyta), brown algae (Phaeophyta) and the red algae (Rhodophyta). Either Freshwater macroalgae or marine macroalgae (kelp or

seaweed) could be used for solar energy conversion and biofuel production [3]. Macroalgae received a large amount of attention as a biofuel feedstock due to its prolific growth in natural habitat of freshwater system, eutrophic coastal water fouling beaches and coastal waterways. Generally, macroalgae (red, brown, and green) are obtained from natural and cultivated resources.

*Spirogyra* sp. (Tao) is freshwater macroalgae, available in the north and northeast of Thailand. It contain high amount of chemical components including carbohydrates, fat, proteins and mineral substances [4]. It is a genus of filamentous freshwater green algae of the Division Chlorophyta order Zygnematales Family Zygnemataceae., named for the helical or spiral arrangement of the chloroplasts that is diagnostic. It is commonly found in freshwater areas and there are more than 400 species of *Spirogyra* in the world [5]. They grow in the standing water of clean to moderate quality, clear water with the turbidity not exceeding 10 NTU, temperature 15-27°C and pH 6-7.8. Regarding biofuel production, algae can provide different types of biofuels, including: biodiesel (from algal fatty acids); ethanol (produced by fermentation of starch); hydrogen (produced biologically); and methane (produced by anaerobic digestion of algal biomass). From an environmental and resource-efficiency perspective biogas has several advantages in comparison to other biofuels. Hughes et al. [6] suggested that energy conversion via anaerobic digestion was successful as the biochemical composition of macroalgae makes it an ideal feedstock. The production of biogas via anaerobic digestion (AD) is the most feasible and cost-effective route to an energy product [7], [8].

Biogas is composed of 40-70% methane (CH<sub>4</sub>), 60-30% carbon dioxide (CO<sub>2</sub>), 100-3000 ppmv hydrogen sulfide (H<sub>2</sub>S) and water, other trace gas compounds and other impurities. Since the main components of biogas are CH<sub>4</sub>, CO<sub>2</sub> and H<sub>2</sub>S. Purification and upgrading of the gas is necessary because purified biogas provides reductions in green house gas emissions as well as several other environmental benefits when used as a vehicle fuel. Reducing CO<sub>2</sub> and H<sub>2</sub>S content will significantly improve the quality of biogas. Various technologies have been developed and available for biogas impurity removal; and biological processes are environmentally friendly and feasible. Furthermore, microalgae are abundant and omnipresent. Biogas purification using microalgae involved photosynthetic ability in the removal of the impurities present in biogas. In Thailand, there is plenty of freshwater natural resource available. In this study, we utilized the natural recourses and obtain algal biomass from the resource directly for biogas production. It could be reduce the cultivation and production cost. In the same time, this approach could be helpful to

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decrease algae degradation and pollution in the water body. In addition, it could reduce the algal bloom. However, biogas is containing with impurities. Biogas impurity removals through microalgal system are beneficial for environment and economical aspects [9]. It could be save environment effects such as green house gases and same time it could reduce the chemical cost and other related purification material cost. Therefore, this study focused on the development in AD techniques for macroalgae as biogas producer and using microalgae as biogas purification. The main objectives are (1) to utilize the natural recourses and evaluation of algae growth environment, (2) to investigate the biogas production potential from freshwater macroalgae (*Spirogyra ellipsozona*) and (3) to apply the biogas purification through microalgal (*Chlorella vulgaris*) biological purification process.

## II. MATERIALS AND METHODS

### A. Plant materials (macroalgae)

*Spirogyra ellipsozona* biomass was collected from the slow running fresh water stream at Tumbon Pang Yang (19° 18'42.41" N; 98° 48'44.11" and elevation 722 m), Mae Taeng district, Thailand and transported to the Energy Research Center, Maejo University, Sansai, Chiang Mai-50290, Thailand. Species identification and morphological details were presented in our previous study [10], [11]. Figure 1 demonstrated that study site, material collection, harvesting and drying through solar dryer for biochemical analysis. Samples of macroalgae biomass were collected by hand (traditional method) directly from the stream. Directly after acquisition macroalgae biomass was rinsed with tap water to remove sand and other pollutants.



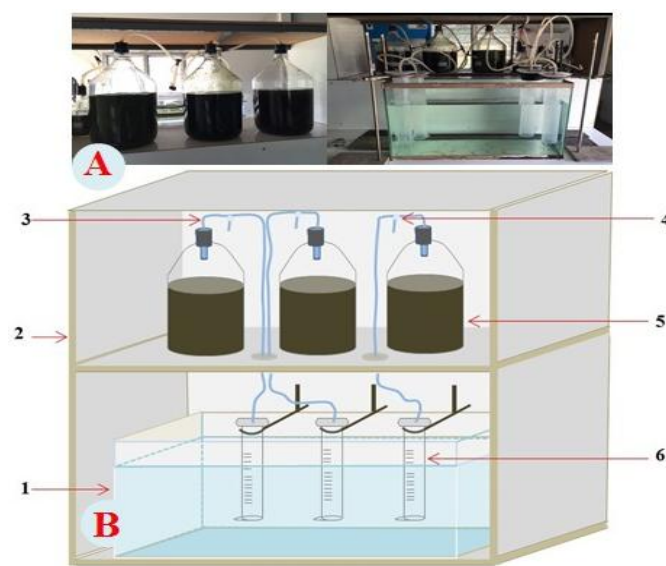
**Figure 1** – Study site and plant material collection: (A) slow running fresh water stream, (B) traditional algae collection, (C), harvested algae, (D) solar dryer for biomass drying.

### B. Microalgae collection for biogas purification

The cultured microalgae, *C. vulgaris* were obtained from Energy Research Center, Maejo University, Sansai, Chiang Mai-50290, Thailand. The algae were cultivated using open type cement pond and low cost artificial medium which is named as Rameshprabu medium.

### C. Experimental procedure

The bioreactor system consists of flasks of 5000 ml in capacity. The equipment is constituted of: valves, quick release tubing connectors, plastic pipes and gas collector, shown in figure 2. To preserve anaerobic conditions, nitrogen has been flushed for 2 min into the reactors to clear up any residual trace of oxygen from within the flasks and pipes. Water-baths were used to keep the reactors at a mesophilic temperature in the laboratory. A biogas analyzer was used to verify anaerobic conditions were created correctly when preparing the reactors and to analyze the biogas biochemical composition. The experimental set up and methodologies are followed our previous studies [10], [11]. The purpose of the first experiment is to identify whether a benefit in room temperature system for biogas production. The anaerobic assays were conducted in 5000 mL duran bottles (triplicate reactor) containing 400 mL of inoculum and 1000 g of fresh *S. ellipsozona* and remaining make up with double distilled water. The total working volume is 4000 mL. After inoculation, all batch reactors were purged with nitrogen gas to create an anaerobic condition. Triplicate, 5000 mL fermenters were incubated in the room temperature (assumed as mesophilic conditions). Production of biogas was monitored by measuring the overpressure in the bottle headspace at time intervals depending on the production of biogas. Three digesters have been prepared with the exact amount of inoculums used. The anaerobic inoculum was obtained from a working anaerobic digester at Energy Research Center, Maejo University.



**Figure 2** – A) Batch system of macroalgae biogas production B) Schematic view of the experimental set up during anaerobic digestion (1) water tank 2) protection wooden box 3) gas transfer tube 4) gas sampling port 5) digester (5000ml) 6) gas measuring cylinder

The inoculums characteristics including TS, VS, COD were  $296.1 \pm 0.05$  mg/L,  $158.5 \pm 1.15$  mg/L and  $1241.6 \pm 2.01$  mg/L, respectively; along with alkalinity of  $136.4 \pm 0.04$  mg/L as  $\text{CaCO}_3$ , VFA of  $136.4 \pm 0.25$  mg $\text{CH}_3\text{COOH/L}$  and pH was  $6.66 \pm 0.03$  [12]. Five liter batch fermenters were

incubated at room temperature conditions for 70 days. The digesters were shaken two or three times everyday to prevent the formation of surface crust which may prevent contact between microorganisms and the substrate.

#### D. Analytical Methods

All the indices including chemical oxygen demand (COD), total nitrogen (TN), total phosphorous (TP), Total solids (TS) and volatile solids were continuously monitored throughout the study, following the standard protocols of APHA [13]. Metrohm 774 pH-meter was used in all pH measurements. Fatty acid content was performed by GC-MS analysis. Protein, carbohydrates, lipids determination procedures were adopted from Tipnee et al. [14]. Elemental composition (C, H, N, O, S) was analyzed using the element analyzer (Perkin-Elmer 2004). Moisture content of raw materials was determined following the procedure given in ASTM Standard D 4442-07. The residual sample in the crucible was heated without lid in a muffle furnace at  $700 \pm 50^\circ\text{C}$  for one half hour. The crucible was then taken out, cooled first in air, then in desiccators and weighed. Heating, cooling and weighing was repeated, till a constant weight obtained. Total fat, ash, moisture, fiber contents and volatile fatty acids (VFA) were determined using AOAC official method [15]. The composition of biogas ( $\text{CH}_4$ ,  $\text{CO}_2$ ,  $\text{H}_2\text{S}$ ,  $\text{H}_2$  and  $\text{O}_2$ ) was measured using a biogas analyzer (GFM 416 series, UK). All the values or readings are the result of mean of three replicates. Data was reported as mean  $\pm$  standard deviation (SD). Statistical analyses were performed using Microsoft Excel. The statistical significances were achieved when  $p < 0.05$ .

### III. RESULTS AND DISCUSSION

#### Feedstock characteristics

Results of physical and chemical composition of the *S. ellipsozpora* after harvested and biomass showed distinct differences in their chemical composition. The fixed carbon, volatile matter, moisture, ash, pH, carbon, hydrogen, oxygen and nitrogen contents were in *S. ellipsozpora*, 13.38%, 66.16%, 8.27%, 15.02%, 7.4, 41.87%, 6.00%, 35.77% and 0.43 %, respectively. The macromolecules of carbohydrate, protein, lipid and fiber contents were 56.43%, 21.60%, 9.81%, and 6.50%, respectively. In this study, the sample content of total solids (TS) and volatile solids (VS) was measured; the results were average as 16622 mg/kg and 13959 mg/kg, respectively. The average was COD 14236 mg/L. Consequently *S. ellipsozpora* has plenty of nutrients for biogas production process; this macroalgae is suitable to be used as energy crops for biogas production. .

#### Macroalgae, *Spirogyra ellipsozpora* to biogas: anaerobic digestion

Macroalgae can be converted to biofuels by various processes including thermal treatment [16] and fermentation [17], [18] but the most direct route to obtaining biofuel from macroalgae is via its anaerobic digestion to biogas (~ 60% methane). Methane can be used to produce heat and electricity or compressed for use as a transport fuel. Since this time there have been developments in AD technology and an

enormous increase in its use Hughes et al. [18]. The study results of biogas and methane production from macroalgae, *S. ellipsozpora* presented in figure 3 and 4. Without any pretreatment methane content was achieved 64.67 %. Our studies agreed with Hughes et al. [18] and also confirmed better results; on 70<sup>th</sup> day  $\text{CO}_2$  level was 31.5 % and  $\text{H}_2\text{S}$  was 578 ppm (i.e. 0.0578%). For the comparison of freshwater macroalgae biogas production and methane content from *S. ellipsozpora* was much higher than *Spirogyra neglecta*, *Chara fragilis* and *Cladophora glomerata*, which was reported by Baltrėnas and Misevičius [19].

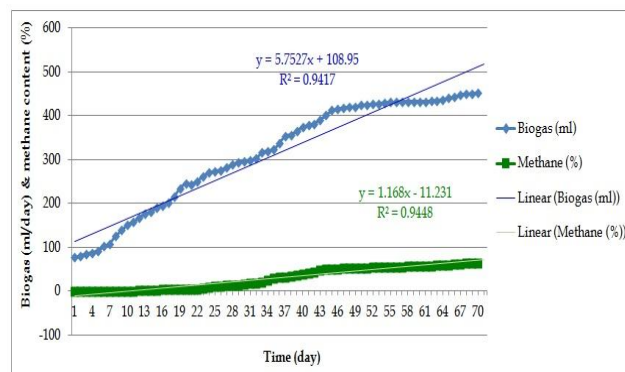


Figure 3 – biogas and methane production from macroalgae, *S. ellipsozpora*.

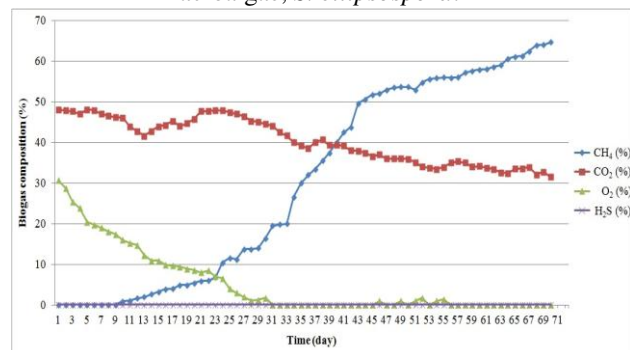


Figure 4 – biogas composition of macroalgae, *S. ellipsozpora* during anaerobic digestions.

#### Biogas purification through biological process

$\text{CO}_2$  Microalgae can fix  $\text{CO}_2$  using solar energy with efficiency ten times greater than terrestrial plants [20], [21]. Therefore, we require the rapid development of bio-carbon-fixation technology to eliminate the adverse effects of  $\text{CO}_2$ , to transfer atmospheric  $\text{CO}_2$  through the carbon cycle and to promote carbon balancing ecologically. The most studies in the literature concerned the maximum  $\text{CO}_2$  uptake rate by the artificial photo-bioreactors [22],[23]. Among those techs, bio-eco-technology is the most natural and ecological way to accomplish the designed targets by the utilization of “self-designed” bio-functions of nature [24],[25]. Accordingly, algae production has a great potential for  $\text{CO}_2$  bio-fixation process and deserves a close look.

Biogas purification/scrubbing using algae involved the use of algae’s photosynthetic ability in the removal of the impurities (mainly  $\text{CO}_2$  and  $\text{H}_2\text{S}$ ) present in biogas, leaving a purified biogas containing almost pure methane, which could be used for energy generation. Biological purification technology is worth examining because has double impact.

The method about removing CO<sub>2</sub> from biogas by microalgal culturing using the biogas effluent as nutrient medium and effectively upgrade biogas also simultaneously reduce the biogas effluent nutrient [26]. Using biogas as a source of carbon dioxide has two main advantages: the biomass production costs are reduced and the produced biomass does not contain harmful compounds, which can occur in flue gases. Hendroko et al. [27] verified exhibit that microalgae (*Scenedesmus* sp.) in laboratory experiments using biogas slurry as growing medium and biogas are given periodically generate 21% of CO<sub>2</sub> compared with 24% of controls. There are several authors [26], [27], [28] reported that *Arthrospira* sp, *Chololera vulgaris* SAG 211-11b, *Chlorella* sp. MM-2, *Chlorella* sp. MB-9, *Chlorella vulgaris* ARC1, *Chlamydomonas* sp. dan *Scenedesmus* sp. was a positive synergy with biogas. The productivity of the system with Zarrouk media and biogas almost 5 times higher than that for the same media without biogas when piggery waste was used, the utilization of biogas brings a productivity gain of about 2–5 times higher [27].

Kao et al. [29] demonstrates that the microalga *Chlorella* sp. MB-9 was a potential strain which was able to utilize CO<sub>2</sub> for growth when aerated with desulfurized biogas (H<sub>2</sub>S<50ppm) produced from the anaerobic digestion of swine wastewater. The demonstrated system can be continuously used to upgrade biogas by utilizing a double set of photobioreactor systems and a gas cycle-switching operation. Furthermore, they demonstrated that the efficiency of CO<sub>2</sub> capture from biogas could be maintained at 50% on average, and the CH<sub>4</sub> concentration in the effluent load could be maintained at 80% on average, i.e., upgrading was accomplished by increasing the CH<sub>4</sub> concentration in the biogas produced from the anaerobic digestion of swine wastewater by 10%.

Some literatures mentioned about the cultivation microalgae using biogas as CO<sub>2</sub> provider. Kao et al. (2012a) used biogas that contained 20±2% CO<sub>2</sub> for *Chlorella* sp. culture with variation of light intensity which was at cloudy and at sunny day. Kao et al. [30] used biogas that contained 20±1% CO<sub>2</sub> for *Chlorella* sp. culture with variation flow rate of biogas which was 0.05; 0.1; 0.2; 0.3 vvm. Doušková et al. (2010) investigated the potential of biogas as CO<sub>2</sub> provider for *Chlorella vulgaris*; and optimization of biogas production from distillery stillage is described. The growth kinetics of microalgae *Chlorella* sp. consuming biogas or mixture of air and CO<sub>2</sub> in the concentration range of 2–20% ( v/v) (simulating a flue gas from biogas incineration) in laboratory-scale photo-bioreactors. It was proven that the raw biogas (even without the removal of H<sub>2</sub>S) could be used as a source of CO<sub>2</sub> for growth of microalgae. The growth rate of microalgae consuming biogas was the same as the growth rate of the culture grown on a mixture of air and food-grade CO<sub>2</sub>. Several species of algae can metabolize H<sub>2</sub>S. Using a biological system to remove H<sub>2</sub>S has similar benefits to using one to remove CO<sub>2</sub>: lower upkeep costs, more environmentally sustainable and non-hazardous waste.

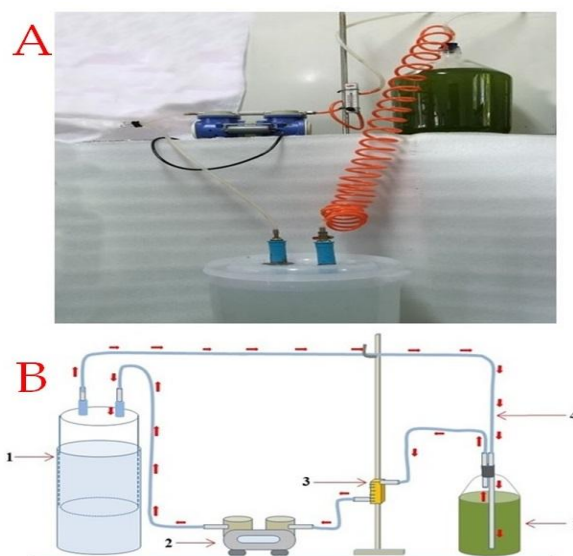
Furthermore, Tongprawhan et al. [31] used oleaginous microalgae to capture CO<sub>2</sub> from biogas for improving methane content and simultaneously producing lipid. They screened several microalgae for identify their ability to grow and produce lipid using CO<sub>2</sub> in biogas. Finally, they reported a marine *Chlorella* sp. was the most suitable strain for

capturing CO<sub>2</sub> and producing lipid using biogas (50% v/v CO<sub>2</sub> in methane) as well as using 50% v/v CO<sub>2</sub> in air. Sumardiono et al. [32] established to evaluate the design of the photobioreactor system for purifying biogas through the culturing of microalgae. This system represented a simple promising way for the current forthcoming technologies of biogas purification. It helps to decrease the concentration of CO<sub>2</sub> in biogas concomitantly producing microalgae biomass. The microalgae *Nannochloropsis* is able to use CO<sub>2</sub> from biogas produced from the anaerobic digestion of tannery sludge. The results show that cultivation of microalgae under the biogas to scrub out CO<sub>2</sub> and promote enrichment of methane in the biogas in this work and obtained scrubbing of 27% from 30%.

The biocapture of CO<sub>2</sub> by microalgae can be applied to improve the quality of biogas by reducing the CO<sub>2</sub> content as this would lead to an increase in the methane content [33]. The microalgae *Chlorella* sp. was analyzed in terms of conditioning biogas. As a result the biogas components CO<sub>2</sub> and H<sub>2</sub>S could be reduced up to 97.07% and 100%, respectively. Also an increase of microalgae cell count could be documented, which provides interesting alternatives for the production of algae ingredients. Consequently, the algae biological purification is an alternative to other biogas purification methods.

#### Biogas purification through microalgae *Chlorella vulgaris*

The entire unit of biogas purification through microalgae *C. vulgaris* system and schematic view of the experimental set up during biogas upgrading processes were shown in figure 5. The microalgal culture obtained directly from the open pond system which was grown near this experimental zone. Ten liter of *C. vulgaris* cultures was used through 10 L duran bottles. The biogas obtained from macroalgae fermenter which is involved in the study. The macroalgae, *S. ellipospora* biogas production processes are above mentioned.



**Figure 5–** A) biogas purification system through microalgae, *C. vulgaris* B) schematic diagram of set up during biogas upgrading 1) biogas storage container 2) air pump 3) air flow meter 4) biogas pumping direction and 5) microalgae unit.

**Table 1**– Test and evaluation of the system performance through biological upgrading.

Component	Biogas production analysis	
	Before the system improve	After the system improvement
CH <sub>4</sub>	64.67%	82.05%
CO <sub>2</sub>	31.5 %	17.08%
O <sub>2</sub>	0%	1.11%
H <sub>2</sub> S	578 ppm	Less than 0.01 ppm

Photoautotrophic The purification process was continued 8 hours. The results were presented in table 1. After purification, the CH<sub>4</sub> content has improved gigantically. Also CO<sub>2</sub> and H<sub>2</sub>S amounts were reduce a lot. Due to algal photosynthesis process O<sub>2</sub> was slightly increased. Reducing CO<sub>2</sub> and H<sub>2</sub>S content will significantly improve the quality of biogas. However this study suggested that long time purification process needed. For biogas impurity removal, biological processes are environmentally friendly and feasible. Consequently biogas purification using algae involved the use of algae's photosynthetic ability in the removal of the impurities present in biogas. Further our establishment study, *C. vulgaris* applicable for biogas purification.

#### IV. CONCLUSIONS

The cultivation of the microalga, *C. vulgaris* utilized low cost artificial medium which named as Rameshprabu medium. The medium was prepared through rice fertilizer, rice bran, fish meal, lime and urea. This medium was named as, Rameshprabu medium. The aim of the current work was to evaluate the potential of the green alga *C. vulgaris* as a cheap renewable energy source in term of biofuels. The algae grew faster, providing higher productivities of biomass, lipids, carbohydrates and proteins. Furthermore, *C. vulgaris* biomass production and carbohydrate consumption were enhanced by supplementing the inorganic culture. Photoautotrophic conditions cultivation of *C. vulgaris* can be considered as a feasible strategy to reduce the costs of microalgal biomass production, while also contributing to solve the environmental problem.

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List of publications:

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List of publications:

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